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[Continued on next page]

(54) Title: CENTRAL NERVOUS SYSTEM DAMAGE

AMORCES PCR

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MAG1:  5-  CG GGA TCC ATG ATA TTC CTT ACC ACC CT
              BamH I  Met

MAG2:  5-  TCC C CGC GGC TCG GTG TGC TCC CTG GAA
                  Sac II


TNR1:  5- TCC CC GCG GCA TGT CCA TGT GCC AGT TCA
                Sac II

TNR2:  5-  TT GC GGC CGC TGG AGG GGC AAC TGC TGA
              Not I


NogoN1:  5-TT GCG GCC GCA ATG GAA GAC CTG GAC CAG TCT
              Not I

NogoN2:  5-AAA C TGC AGC CAC TGA GCC CGA GGA GCC CCT
                Pst I


Nogo66-1:  5-AAA CT GCA GCA AGG ATA TAC AAG GGT GT
                 Pst I

Nogo66-2:  5- GC TCT AGA TCA CTT CAG AGA ATC AAC TA
               Xba I   ***
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CONSTRUCTION OBTENUE A PARTIR DE PRODUITS PCR RELIES SEQUENTIELLEMENT

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                Ala Ala Ala      Ala Ala Ala      Ala Ala Ala
5'-GGA TCC(MAG) GCC GCG GCA(TNR) GCG GCC GCA(NogoN) GCT GCA GCA
   BamH I        Sac II           Not I             Pst I


            ***
(Nogo66)TGA TCT AGA-3'
            Xba I
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(57) Abstract: The application provides peptides that interact with the inhibitory domains of the myelin proteins Nogo, TNR and MAG. These may be used in the treatment for CNS damage, and for the development of further treatments. Also provided are methods and materials for immunizing subjects against the inhibitory domains of the myelin proteins, for the treatment for CNS damage.

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Central Nervous System Damage

The invention relates to materials and methods useful in the treatment of, or in the development of treatments for, CNS
5   damage, e.g. spinal cord injury or stroke.

Background

Most tissues in the body, such as skin, liver and peripheral
10   nerve, have a remarkable ability to repair themselves after injury. By contrast, the central nervous system (CNS) – including the brain and the spinal cord – has little innate capacity for repair. When axonal connections are damaged in the adult brain or spinal cord, they show an extremely
15   limited ability to regenerate, even though axons can grow and regenerate efficiently in the embryonic CNS and in the adult peripheral nervous system. Factors that account for the inability of CNS axons to regenerate can be grouped in two categories: intrinsic properties of CNS neurons that
20   may make them incapable of regeneration; and extrinsic factors in the CNS environment that are inhibitory to axonal elongation.

The idea that factors in the CNS environment can prevent
25   regeneration dates back to the early 20th century. Ramon y Cajal observed that the inability of adult CNS neurons to extend axonal processes could be overcome by giving them the permissive environment of a peripheral nerve. Then, about 20 years ago, David and Aguayo showed that retinal neurons
30   could form long projections in peripheral nerve grafts. Later, Schwab discovered that dorsal root ganglion neurons in culture extend their axons across Schwann cells but avoid oligodendrocytes and the fatty myelin sheath (Schwab et al 1993).

These results show that failure to regenerate is not purely
an intrinsic deficit of CNS neurons, and inhibitory factors
in the CNS environment also play an important role. These
5   inhibitory factors are mainly located in the glial scar that
forms at the region of injury and by the myelin that
ensheaths axons in the white matter tracks.

Following CNS injury, the central area of necrosis is
10 infiltrated by glia and other non-neuronal cells, and a
fibrous scar forms. Axons do not extend through the scar
and their growth appears to be inhibited by it. Molecular
components that may contribute to this inhibitory activity
include the extracellular matrix glycoprotein tenascin-R
15 (TN-R) and the myelin-associated neurite outgrowth
inhibitors myelin-associated glycoprotein (MAG) and Nogo.

TN-R

20 TN-R has been implicated in a variety of cell-matrix
interactions involved in the molecular control of axon
guidance and neural cell migration during development and
regeneration of CNS (reviewed by Erickson, 1993; Chiquet-
Ehrismann et al., 1994; Pesheva et al., 2000; 2001). TN-R
25 is the smallest member of the tenascin family and is
composed of four structural motifs: a cysteine-rich segment
at the N-terminus is followed by 4.5 EGF-like repeats. This
region is followed by 9 consecutive fibronectin type III-
like domains and at the C-terminus TN-R is related to the
30 beta- and- gamma-chains of fibrinogen.

TN-R is expressed predominantly by oligodendrocytes during
the onset and early phases of myelin formation, and remains
expressed by some oligodendrocytes in the adult. TN-R is

also expressed in some neurons and interneurons in the
spinal cord, retina, cerebellum, and hippocampus (Fuss et
al., 1991; 1993). TN-R co-localizes with other glial-
derived molecules (i.e. myelin-associated glycoprotein and a

5   phosphacan-related molecule) at high density in the nodes of
Ranvier of CNS myelinated axons (Xiao et al., 1997: Yang et
al, 1999).

TN-R can inhibit or promote neurite outgrowth, depending on

10   the neuronal cell type and the environment in which it is
presented. When TN-R was offered, acting as a sharp
substrate boundary, dorsal root ganglion (DRG), cerebellum
and retinal ganglion neuron growth cones avoided growing on
these molecules; but were not induced to collapse. On the

15   other hand, when TN-R was offered in a mixture with laminin
(which strongly promotes growth of embryonic and adult
axons) as a uniform substrate, DRG growth cones displayed a
collapsed morphology and were able to advance at a faster
rate than on laminin alone.

20
Using several monoclonal antibodies binding to distinct
epitopes on the tenascin molecule, epidermal growth factor-
like (EGF-L) repeats and fibronectin type III homologous
repeats 4-5 were identified to be responsible for growth

25   cone repulsion.

In vitro, outgrowth of embryonic and adult retinal ganglion
cell axons from mouse retinal explants is significantly
reduced on homogeneous substrates of tenascin-R or a

30   bacterially expressed tenascin-R fragment comprising the
EGF-L domain. When both molecules are presented, acting as
a sharp substrate boundary, regrowing adult axons do not
cross into the territory containing tenascin-R or EGF-L.
All in vitro experiments were done in the presence of

3

laminin, suggesting that tenascin-R and EGF-L actively
inhibit axonal growth. Neurites and growth cones were
repelled from areas coated with fragments containing the
EGF-L (the amino-terminal cysteine-rich domain plus the EGF-
5   like repeats), FN (fibronectin) 1-2, FN3-5 and FG
(fibrinogen) domains of TN-R, and EGF-L prevents neurite
outgrowth of hippocampal neurons.

TN-R also induces axonal defasciculation in vitro through
10   the EGF-L domain (Taylor et al., 1993; Xiao et al., 1996,
1997, 1998; Becker et al, 1999; Becker et al, 2000).

After 3-acetylpyridine-induced lesion of the olivocerebellar
system of the adult rat, the density of cells containing TN-
15   R transcripts increased significantly in the inferior
olivary nucleus and in the white matter of the cerebellar
cortex. Immunohistochemical investigations confirmed these
observations at the protein level.

20   After a spinal cord mechanical lesion of rat, TN-R mRNA was
also upregulated (Wintergerst at al, 1997; Deckner et al,
2000).

These findings suggested that the continued overexpression
25   of TN-R in the injured CNS may contribute to the failure of
adult axonal regeneration in vivo.

Tenascin-R is a member of the tenascin family, which play
important roles in cell interactions in the developing
30   nervous system, such as neuronal migration, neuritogenesis,
and neuronal regeneration. Tenascin-R is expressed
predominantly by oligodendrocytes during the onset and early
phases of myelin formation, and remain expressed by some
oligodendrocytes in the adult (Pesheva et. al., 1989; Fuss

et al., 1991,1993; Wintergerst et. al., 1993; Ajemian A. et.
al., 1994). TN-R is a multi-functional molecule (Lochter and
Schachner, 1993; Pesheva et. al., 1993; Taylor et. al.,
1993; Xiao et. al., 1996, 1997,1998), and it has indicated

5   that it is an inhibitory component of myelin extraction.
Xiao have found that, the EGF-L domain of TN-R can inhibit
the neurite outgrowth.

MAG

10

MAG is a transmembrane protein of the immunoglobulin
superfamily expressed by myelinating glial cells of the
central and peripheral nervous systems, where MAG represents
1 and 0.1% of the total myelin proteins, respectively (Heape

15   at al, 1999). MAG is a potent inhibitor of axonal
regeneration and also, depending on the age and type of
neuron, can promote axonal growth. MAG inhibits neurite
outgrowth of retinal, superior cervical ganglion, spinal,
and hippocampal and dorsal root ganglion (DRG) neurons of

20   all postnatal ages, but can enhance neurite outgrowth of
embryonic spinal cord neurons and newborn DRG neurons
(DeBellard at al, 1996; Turnley et al, 1998; Shen et al,
1998; Yang et al, 1999).

25   MAG can also induce growth cone collapse. 60% of axonal
growth cones of postnatal day 1 hippocampal neurons
collapsed when they encountered coated recombinant MAG
(rMAG). Such collapse was not observed with denatured rMAG
(Li et al, 1996). Soluble dMAG (a proteolytic fragment of

30   the extracellular domain of MAG, which is released in
abundance from myelin and found in vivo) and chimeric MAG-Fc
can potently inhibit neurite outgrowth from P6 DRG neurons.
This inhibition was blocked when a MAG monoclonal antibody
was included.

These results indicate that soluble dMAG detected in vivo
could contribute to the lack of regeneration in the
mammalian CNS after injury (Tang et al, 1997; 2001).

5

MAG has two recognition sites for neurons, the sialic acid
binding site at R118 and a distinct inhibition site which is
absent from the first three Ig domains (Tang et al, 1997).

10   MAG is a well characterized member of the immunoglobulin
gene superfamily, and it exerts a robust inhibitory effect
on neurite outgrowth from young cerebellar neurons and adult
dorsal ganglion (DRG) neurons (Mukhopadhyay et al., 1994).
MAG is a membrane protein with 626 amino acids. It has been
15   reported that soluble MAG, which consists of the
extracellular domain, has an inhibitory effect on neurite
outgrowth (Mckerracher et. al., 1994). The extracellular
domain of MAG consists of five Ig-like domains, and it is
demonstrated that the first two Ig-like domains are
20   important for the interaction between MAG and neuronal
membrane, while the other three Ig-like domains might be
involved in the inhibitory effects (Collins et al., 1997).

Nogo

25

Nogo is a high molecular weight integral membrane protein
that localizes to CNS myelin, but not PNS myelin. Nogo has
three isoforms, named Nogo-A, -B and -C, which are generated
by alternative splicing. NI-250 and NI-35 were first
30   identified and named as the 2 isoforms of Nogo; it has now
been established that NI-250 is Nogo-A and NI-35 is Nogo-B.
Nogo is expressed by oligodendrocytes in white matter of the
CNS and is found in the inner and outer leaflets of myelin
and in the endoplasmic reticulum.

In vitro characterization of Nogo has demonstrated its
function as a potent inhibitor of axon elongation. In vivo
neutralization of Nogo activity results in enhanced axonal
5   regeneration and functional recovery following CNS injury as
well as increased plasticity in uninjured CNS fibers. The
monoclonal antibody mAb IN-1 was shown to promote long-
distance regeneration and functional recovery in vivo when
applied to spinal cord-injured adult rat (Chen et al, 2000;
10   GrandPre et al; Merkler et al).

These findings suggest that Nogo may be a major contributor
to the nonpermissive nature of the CNS environment. Two
distinct inhibitory domains of Nogo have been identified: an
15   intracellular amino-terminal domain (NogoN) of Nogo A and a
short 66 residue region (Nogo-66) located between two
hydrophobic domains of the three isoforms, Nogo-A, Nogo-B
and Nogo-C (Chen et al, 2000; GrandPre et al, 2000; Fournier
et al , 2001; Filbin, 2003). These domains are illustrated
20   in Science, Vol 297 (5584), 16 Aug 2002, p. 1132-1134.

Nogo-A is expressed by oligodendrocytes but not by Schwann
cells. It can inhibit axonal extension and collapses dorsal
root ganglion growth cones (GrandPre et. al., 2000). The
25   neurite outgrowth inhibitory activity of Nogo can be
neutralized by monoclonal antibody IN-1, which allows axonal
regeneration and functional recovery after spinal cord
injury (Chen et. al., 2000). Nogo is a membrane protein with
1163 amino acids. The C-terminal tail contains two
30   hydrophobic transmembrane domains separated by a 66-residue
hydrophilic extracellular domain. This 66-residue
extracellular domain can inhibit axon outgrowth (Fournier
et. al., 2001).

Huang et al. (1999) discloses a therapeutic vaccine approach
to stimulate axonal regeneration in the adult spinal cord.

It is an object of the invention to provide materials and
5   methods useful in the treatment of, or in the development of
treatments for, CNS damage, e.g. spinal cord injury, by
overcoming the inhibitory effects of myelin on axonal
regeneration.

10 **Summary of Invention**

In a first general aspect, the inventor proposes using
molecules which interact with the inhibitory domains of
major myelin proteins as an adjunct treatment for CNS
15 damage, and for the development of further treatments.

In a second general aspect, the inventor proposes immunizing
subjects with the inhibitory domains of major myelin
proteins as an adjunct treatment for CNS damage.
20
It may appear impractical to immunize subjects against
myelin antigens, as this approach may induce autoimmune
demyelinating disease, leading to unacceptable side effects.
Also, some myelin antibodies promote remyelination
25 (Rodriguez et al., 1987), which is not of benefit during
axonal regeneration. The inventor's approach is, however,
to base the vaccine on the specific inhibitory portions of
major myelin proteins, instead of whole myelin proteins.
Antibodies raised against the inhibitory portions will block
30 the inhibitory effect of myelin on axonal regeneration.

First aspect

8

Accordingly, the invention provides a peptide, the amino
acid sequence of which consists of an amino acid sequence
selected from the group consisting of:

        YLTQPQS (SEQ ID NO. 1);

5   GSLPHSL (SEQ ID NO. 2);

        TQLFPPQ (SEQ ID NO. 3);

        HSIPDNI (SEQ ID NO. 4);

        HHMPHDK (SEQ ID NO. 5);

        YTTPPSP (SEQ ID NO. 6); and

10   QLPLMPR (SEQ ID NO. 7).

These sequences each represent the deduced amino acid
sequences of several peptides that have been identified by
phage display as being capable of binding to one or more of

15 the neuronal growth inhibitory molecules Nogo (specifically,
the Nogo-66 domain), MAG and TN-R (specifically, TNR-EGFL).

SEQ ID NO. 1 represents the sequence of 43 identical
peptides capable of binding to Nogo-66, and also the

20 sequence of 19 identical peptides capable of binding to MAG.

SEQ ID NO. 2 represents the sequence of 8 identical peptides
capable of binding to Nogo-66.

25 SEQ ID NO. 3 represents the sequence of 18 identical
peptides capable of binding to TNR-EGFL.

SEQ ID NO. 4 represents the sequence of 3 identical peptides
capable of binding to TNR-EGFL.

30

SEQ ID NO. 5 represents the sequence of 1 peptide capable of
binding to TNR-EGFL.

SEQ ID NO. 6 represents the sequence of 1 peptide capable of binding to TNR-EGFL.

SEQ ID NO. 7 represents the sequence of 5 identical peptides
5   capable of binding to MAG.

Of these, SEQ ID NO. 1 has been shown by phage binding to block the inhibitory effects of Nogo-66 and MAG on neuronal cell adhesion in an in vitro assay. Similarly, SEQ ID NO. 3
10   has been shown to block the inhibitory effect of TNR-EGFL. A preferred peptide therefore consists of SEQ ID NO. 1; another preferred peptide consists of SEQ ID NO. 3.

The invention further provides a peptide up to 60 amino
15   acids in length comprising an amino acid sequence selected from the group consisting of:
        YLTQPQS (SEQ ID NO. 1);
        GSLPHSL (SEQ ID NO. 2);
        TQLFPPQ (SEQ ID NO. 3);
20   HSIPDNI (SEQ ID NO. 4);
        HHMPHDK (SEQ ID NO. 5);
        YTTPPSP (SEQ ID NO. 6); and
        QLPLMPR (SEQ ID NO. 7),
    wherein the peptide is capable of binding to Nogo
25   (preferably Nogo-66), MAG and/or TN-R (preferably TNR-EGFL).

Preferably, the peptide is up to 50 amino acids in length, more preferably up to 40, up to 30, up to 25, up to 20, up to 19, up to 18, up to 17, up to 16, up to 15, up to 14, up
30   to 13, up to 12, up to 11, up to 10, up to 9, or up to 8 amino acids in length.

A preferred peptide comprises SEQ ID NO. 1; another preferred peptide comprises SEQ ID NO. 3.

The invention further provides a peptide up to 60 amino
acids in length comprising an amino acid sequence having at
least 5 residues identical with corresponding residues in an
5   amino acid sequence selected from the group consisting of:

        YLTQPQS (SEQ ID NO. 1);

        GSLPHSL (SEQ ID NO. 2);

        TQLFPPQ (SEQ ID NO. 3);

        HSIPDNI (SEQ ID NO. 4);

10   HHMPHDK (SEQ ID NO. 5);

        YTTPPSP (SEQ ID NO. 6); and

        QLPLMPR (SEQ ID NO. 7),

wherein the peptide is capable of binding to Nogo
(preferably Nogo-66), MAG and/or TN-R (preferably TNR-EGFL).

15

Preferred sizes of the peptide are as stated previously.
Peptides having at least 5 residues identical with
corresponding residues of SEQ ID NO. 1, or at least 5
residues identical with corresponding residues of SEQ ID NO.

20   3 are preferred.

Preferably the minimum number of identical residues is 6.

The invention further provides a composition comprising one
25   or more peptides of the invention, together with one or more
pharmaceutically acceptable ingredients.

Preferably the composition is formulated for injection in
vivo, preferably for direct injection into the CNS.

30

The invention further provides a peptide of the invention
for use in a method of treatment. Such use may be in the
treatment of CNS damage, especially spinal cord injury and
stroke.

The invention also provides the use of a peptide of the
invention in the preparation of a medicament for the
treatment of CNS damage, especially spinal cord injury and
5   stroke.

The invention also provides a method for treating CNS
damage, the method comprising administering a peptide of the
invention to a patient at or near a site of CNS damage in
10   the patient. Specifically, the invention provides a method
for treating SCI or stroke, the method comprising
administering to a patient a peptide having an amino acid
sequence that consists of SEQ ID NO. 1 or 3, by direct
injection into a site of SCI or stroke damage in the
15   patient.

The invention also provides the use of a peptide of the
invention and/or a computer-generated model thereof, in the
design of a mimetic capable of binding to one or more of the
20   neuronal growth inhibitory molecules Nogo (preferably Nogo-
66), MAG and/or TN-R (preferably TNR-EGFL).

Similarly, the invention provides a method of designing a
mimetic of a peptide of the invention, the mimetic being
25   capable of binding to one or more of the neuronal growth
inhibitory molecules Nogo (preferably Nogo-66), MAG and/or
TN-R (preferably TNR-EGFL), said method comprising:
     (i)   analysing a peptide of the invention that is
capable of binding to one or more of the neuronal growth
30   inhibitory molecules Nogo (preferably Nogo-66), MAG and/or
TN-R (preferably TNR-EGFL) to determine the amino acid
residues essential and important for the activity to define
a pharmacophore; and

12

(ii) modelling the pharmacophore to design and/or
screen candidate mimetics having the biological activity.

5   Preferably the method and/or use includes a step of assaying
binding of a candidate mimetic to Nogo (preferably Nogo-66),
MAG and/or TN-R (preferably TNR-EGFL) in vitro. Having
identified a candidate mimetic that is capable of such in
vitro binding, the candidate mimetic is preferably optimised
for in vivo use. Following such optimisation, the optimised
10   mimetic is preferably formulated together with one or more
pharmaceutically acceptable ingredients.

The invention further provides a bacteriophage which
expresses a fusion protein consisting of a peptide and a
15   bacteriophage coat protein, such that the peptide is
displayed on the surface of the bacteriophage virion,
wherein the peptide is up to 60 amino acids in length and
comprises an amino acid sequence having at least four
residues identical with corresponding residues of an amino
20   acid sequence selected from the group consisting of:
         YLTQPQS (SEQ ID NO. 1);
         GSLPHSL (SEQ ID NO. 2);
         TQLFPPQ (SEQ ID NO. 3);
         HSIPDNI (SEQ ID NO. 4);
25   HHMPHDK (SEQ ID NO. 5);
         YTTPPSP (SEQ ID NO. 6); and
         QLPLMPR (SEQ ID NO. 7).

Preferably the peptide of the invention is up to 50 amino
30   acids in length, more preferably up to 40, up to 30, up to
25, up to 20, or up to 15 amino acids in length. Still more
preferably, the peptide of the invention is 8-12 amino acids
in length, more preferably 6-10. Preferably the minimum
number of identical residues is 5 or 6.

The invention further provides a screening method for
peptides capable of binding to Nogo (preferably Nogo-66),
MAG and/or TN-R (preferably TNR-EGFL), the method

5   comprising:
         providing bacteriophages of the invention, respectively
expressing different peptides; and
         screening the bacteriophages for the ability to bind to
Nogo (preferably Nogo-66), MAG and/or TN-R (preferably TNR-

10   EGFL).

Bacteriophages which are identified as being capable of
binding to Nogo (preferably Nogo-66), MAG and/or TN-R
(preferably TNR-EGFL), or the peptides they display, may

15   then be screened for the ability to block the inhibitory
effects of Nogo (preferably Nogo-66), MAG and/or TN-R
(preferably TNR-EGFL) on neuronal cell adhesion in an in
vitro assay. Following the identification of a peptide (or
phage that displays a peptide) that is capable of blocking

20   the inhibitory effects of Nogo (preferably Nogo-66), MAG
and/or TN-R (preferably TNR-EGFL) on neuronal cell adhesion
in an in vitro assay, the peptide is preferably formulated
with one or more pharmaceutically acceptable ingredients for
administration in vivo.

25
The invention further provides a method of searching for
factors that are likely to reduce the inhibitory effect of
TN-R, MAG and/or Nogo, the method comprising interrogating
a sequence database to identify polypeptides, or nucleic

30   acids that encode polypeptides, that comprise an amino acid
sequence having at least 5 residues identical with
corresponding residues in an amino acid sequence selected
from the group consisting of:
         YLTQPQS (SEQ ID NO. 1);

          GSLPHSL (SEQ ID NO. 2);

          TQLFPPQ (SEQ ID NO. 3);

          HSIPDNI (SEQ ID NO. 4);

          HHMPHDK (SEQ ID NO. 5);

5   YTTPPSP (SEQ ID NO. 6); and

          QLPLMPR (SEQ ID NO. 7).

The database is preferably a cDNA database. It may be an EST
database. Preferably it is a database of sequences

10 expressed in mammalian CNS. Less specific databases may be
used, although this may generate more false positive
results.

The invention further provides a method of searching for

15 factors that are likely to reduce the inhibitory effect of
TN-R, MAG and/or Nogo, the method comprising screening a
cDNA library with an oligonucleotide probe which is capable
of hybridising under stringent conditions with a nucleic
acid sequence that encodes an amino acid sequence having at

20 least 5 residues identical with corresponding residues in an
amino acid sequence selected from the group consisting of:

          YLTQPQS (SEQ ID NO. 1);

          GSLPHSL (SEQ ID NO. 2);

          TQLFPPQ (SEQ ID NO. 3);

25   HSIPDNI (SEQ ID NO. 4);

          HHMPHDK (SEQ ID NO. 5);

          YTTPPSP (SEQ ID NO. 6); and

          QLPLMPR (SEQ ID NO. 7).

30 The cDNA library is preferably mammalian, more preferably
human. The library is preferably derived from CNS tissue.

Each of the two preceding methods preferably further
includes a step, following the identification of a candidate

polypeptide, or nucleic acid encoding the candidate
polypeptide, of testing the polypeptide for the ability to
reduce the inhibitory effect of TN-R, MAG and/or Nogo.
Preferred further steps are as indicated above.

5

Second aspect

In this aspect, the invention provides nucleic acid vector
comprising nucleic acid encoding one or more polypeptide

10   domains selected from the group consisting of:
          (a) the N-terminal (NogoN) domain of Nogo-A;
          (b) the extracellular loop (Nogo66) of Nogo-B (also
     present in Nogo-A and Nogo-C);
          (c) the third to fifth immunoglobulin-like repeats of

15   MAG; and
          (d) the EGF-like domain of TN-R.

The TN-R EGF-L domain has been identified in Xiao et al.
(1996) as being capable of inhibiting neurite outgrowth in

20   vitro of a neuronal cell line and primary neurons.

The vector will include control sequences necessary for the
expression of said nucleic acid in mammalian cells.
Preferably the vector is a commercially available vaccine

25   vector, into which said nucleic acid has been inserted.
Suitable and preferred commercially available vaccine
vectors include the pcDNA 3.1 family of vectors, especially
pcDNA 3.1$^+$, and pVAX1 (all from Invitrogen, San Diego,
California, US).

30

Usually the nucleic acid will be DNA.

Preferably the nucleic acid encodes at least two of the
domains, more preferably at least three, more preferably it

encodes all four. The nucleic acid may encode a plurality
of copies of any one or more of the domains.

Where the nucleic acid encodes more than one domain, the
5 domains are preferably expressed as a fusion polypeptide.
Preferably the domains are separated from one another by
flexible linkers (preferably poly-Ala linkers, e.g. Ala$_3$
linkers) to facilitate correct folding of the domains.

10 Of the proteins Nogo A, Nogo B, TN-R and/or MAG (and/or
preferably Nogo-C), the vector is preferably capable of
expressing substantially only the domains indicated. NogoN
is a domain of the Nogo A isoform. Nogo 66 is a domain
found in all three isoforms, Nogo-A, Nogo-B and Nogo-C. In
15 particular, the vector is preferably incapable of expressing
other epitope-containing portions of the proteins. Of the
proteins Nogo A, Nogo B, TN-R and/or MAG (and/or preferably
Nogo-C), the vector preferably expresses no more than 20%,
more preferably no more than 15%, no more than 12%, no more
20 than 10%, no more than 8%, no more than 6%, no more than 5%,
no more than 4%, no more than 3%, no more than 2% or no more
than 1% of the protein lying outside the domains listed
above. This is particularly preferred for vectors
containing a domain or domains of only one of the proteins.
25
The amino acid sequences of Nogo A, Nogo B, Nogo C, TN-R and
MAG are available from GenBank, for example under the
following accession numbers:

30   MAG - X05301 (GI 56611)
         TN-R - Z67996 (GI 1261914)
         Nogo B - AJ251384 (GI 9408097)
         Nogo A - AF320999 (GI 11878297)
         Nogo C - CAB99250 (GI 9408100)

The domains preferably have the amino acid sequences shown below, i.e. for domain (c), the 508 residue amino acid sequence of MAG (1-508); for domain (d), the 205 residue amino acid sequence of TNR (125-329); for domain (a), the 185 amino acid sequence of NogoN (1-185); and for domain (b), the 66 amino acid sequence of Nogo66 (823-888).

MAG (1-508)

```
MIFLTTLPLF WIMISASRGG HWGAWMPSSI SAFEGTCVSI PCRFDFPDEL
RPAVVHGVWY FNSPYPKNYP PVVFKSRTQV VHESFQGRSR LLGDLGLRNC
TLLLSTLSPE LGGKYYFRGD LGGYNQYTFS EHSVLDIINT PNIVVPPEVV
AGTEVEVSCM VPDNCPELRP ELSWLGHEGL GEPTVLGRLR EDEGTWVQVS
LLHFVPTREA NGHRLGCQAA FPNTTLQFEG YASLDVKYPP VIVEMNSSVE
AIEGSHVSLL CGADSNPPPL LTWMRDGMVL REAVAESLYL DLEEVTPAED
GIYACLAENA YGQDNRTVEL SVMYAPWKPT VNGTVVAVEG ETVSILCSTQ
SNPDPILTIF KEKQILATVI YESQLQLELP AVTPEDDGEY WCVAENQYGQ
RATAFNLSVE FAPIILLESH CAAARDTVQC LCVVKSNPEP SVAFELPSRN
VTVNETEREF VYSERSGLLL TSILTLRGQA QAPPRVICTS RNLYGTQSLE
LPFQGAHR (SEQ ID NO:8)
```

TNR (125-329)

```
    CPCASS AQVLQELLSR IEMLEREVSV LRDQCNANCC QESAATGQLD
YIPHCSGHGN FSFESCGCIC NEGWFGKNCS EPYCPLGCSS RGVCVDGQCI
CDSEYSGDDC SELRCPTDCS SRGLCVDGEC VCEEPYTGED CRELRCPGDC
SGKGRCANGT CLCEEGYVGE DCGQRQCLNA CSGRGQCEEG LCVCEEGYQG
PDCSAVAPP (SEQ ID NO:9)
```

NogoN (1-185)

```
MEDLDQSPLV SSSDSPPRPQ PAFKYQFVRE PEDEEEEEEE EEEDEDEDLE
ELEVLERKPA AGLSAAPVPT APAAGAPLMD FGNDFVPPAP RGPLPAAPPV
APERQPSWDP SPVSSTVPAP SPLSAAAVSP SKLPEDDEPP ARPPPPPPAS
VSPQAEPVWT PPAPAPAAPP STPAAPKRRG SSGSV (SEQ ID NO:10)
```

Nogo66 (823-888)

```
    RIYKGVIQ AIQKSDEGHP FRAYLESEVA ISEELVQKYS NSALGHVNCT
IKELRRLFLV DDLVDSLK (SEQ ID NO:11)
```

The vector preferably encodes a polypeptide having the amino acid sequence MAG(1-508)-Ala_n-TNR(125-329)-Ala_n-NogoN(1-

185)-Ala$_n$-Nogo66(823-888) (SEQ ID NO:12), where Ala$_n$

represents an polyalanine linker. n is preferably 3.

The domains are preferably encoded by the following

5   sequences, i.e. for domain (c), the 508 codon nucleic acid

sequence of MAG (126-1649); for domain (d), the 205 codon

nucleic acid sequence of TNR (454-1068); for domain (a), the

185 codon nucleic acid sequence of NogoN (1-555); and/or for

domain (b), the 66 codon nucleic acid sequence of Nogo66

10   (2467-2664):

MAG (126-1649)

15   atgat attccttacc accctgcctc tgttttggat aatgatttca
     gcttctcgag gggggcactg gggtgcctgg atgccctcgt ccatctcagc
     cttcgagggc acgtgtgtct ccatcccctg ccgtttcgac ttcccggatg
     agctcagacc ggctgtggta catggcgtct ggtatttcaa cagtccctac
     cccaagaact acccgccagt ggtcttcaag tcccgcacac aagtggtcca
20   cgagagcttc cagggccgta ccgcctgtt gggagacctg ggcctacgaa
     actgcaccct gcttctcagc acgctgagcc ctgagctggg agggaaatac
     tatttccgag gtgacctggg cggctacaac cagtacacct tctcggagca
     cagcgtcctg gacatcatca acacccccaa catcgtggtg cccccagaag
     tggtggcagg aacggaagta gaggtcagct gcatggtgcc ggacaactgc
25   ccagagctgc gccctgagct gagctggctg ggccacgagg ggctagggga
     gcccactgtt ctgggtcggc tgcgggagga tgaaggcacc tgggtgcagg
     tgtcactgct acacttcgtg cctactagag aggccaacgg ccaccgtctg
     ggctgtcagg ctgccttccc caacaccacc ttgcagttcg agggttacgc
     cagtctggac gtcaagtacc ccccggtgat tgtggagatg aattcctctg
30   tggaggccat tgagggctcc cacgtcagcc tgctctgtgg ggctgacagc
     aacccgccac cgctgctgac ttggatgcgg gatgggatgg tgttgaggga
     ggcagttgct gagagcctgt acctggatct ggaggaggtg accccagcag
     aggacggcat ctatgcttgc ctggcagaga tgcctatgg ccaggacaac
     cgcacggtgg agctgagcgt catgtatgca ccttggaagc ccacagtgaa
35   tgggacggtg gtggcggtag aggggggagac agtctccatc ctgtgttcca
     cacagagcaa cccggaccct attctcacca tcttcaagga aagcagatc
     ctggccacgg tcatctatga gagtcagctg cagctggaac tccctgcagt
     gacgcccgag gacgatgggg agtactggtg tgtagctgag aaccagtatg
     gccagagagc caccgccttc aacctgtctg tggagtttgc tcccataatc
40   cttctggaat cgcactgtgc agcggccaga gacaccgtgc agtgcctgtg
     tgtggtaaaa tccaacccgg aaccctccgt ggcctttgag ctgccttccc
     gcaacgtgac tgtgaacgag acagagaggg agtttgtgta ctcagagcgc
     agcggcctcc tgctcaccag catcctcacg ctccggggtc aggcccaagc
     cccaccccgc gtcatttgta cctccaggaa cctctacggc acccagagcc
45   tcgagctgcc tttccaggga gcacaccga (SEQ ID NO:13)

19

This sequence commences with a start codon; if any other
sequence is used at the 5' end of the nucleic acid, a start
codon will be required. It is of course a matter of routine
to engineer this into any nucleic acid sequence of interest,
5   though it will be noted that Nogo(1-555) also commences with
a start codon.

TNR (454-1068)

10   tgtccat gtgccagttc agcccaggtg ctgcaggagc tgctgagccg
gatcgagatg ctggagaggg aggtgtcggt gctgcgagac cagtgcaacg
ccaactgctg ccaagaaagt gctgccacag gacaactgga ctatatccct
cactgcagtg gccacggcaa ctttagcttt gagtcctgtg ctgcatctg
caacgaaggc tggtttggca agaattgctc ggagccctac tgcccgctgg
15   gttgctccag ccggggggtg tgtgtggatg gccagtgcat ctgtgacagc
gaatacagcg gggatgactg ttccgaactc cggtgcccaa cagactgcag
ctcccggggg ctctgcgtgg acggggagtg tgtctgtgaa gagccctaca
ctggcgagga ctgcagggaa ctgaggtgcc ctggggactg ttcggggaag
gggagatgtg ccaacggtac ctgtttatgc gaggagggct acgttggtga
20   ggactgcggc cagcggcagt gtctgaatgc ctgcagtggg cgaggacaat
gtgaggaggg gctctgcgtc tgtgaagagg ctaccaggg ccctgactgc
tcagcagttg cccctcca (SEQ ID NO:14)

NogoN (1-555)

25
atggaagacc tggaccagtc tcctctggtc tcgtcctcgg acagcccacc
ccggccgcag cccgcgttca agtaccagtt cgtgagggag cccgaggacg
aggaggaaga agaggaggag gaagaggagg acgaggacga agacctggag
gagctggagg tgctggagag gaagcccgcc gccgggctgt ccgcggcccc
30   agtgcccacc gcccctgccg ccggcgcgcc cctgatggac ttcggaaatg
acttcgtgcc gccggcgccc cggggacccc tgccggccgc tcccccccgtc
gccccggagc ggcagccgtc ttgggacccg agccggtgt cgtcgaccgt
gcccgcgcca tccccgctgt ctgctgccgc agtctcgccc tccaagctcc
ctgaggacga cgagcctccg gcccggcctc ccctcctcc cccggccagc
35   gtgagccccc aggcagagcc cgtgtggacc ccgccagccc cggctcccgc
cgcgccccc tccaccccgg ccgcgcccaa gcgcaggggc tcctcgggct
cagtg (SEQ ID NO:15)

Nogo66 (2467-2664)
40
     agga tatacaaggg tgtgatccaa gctatccaga aatcagatga
aggccaccca ttcagggcat atctggaatc tgaagttgct atatctgagg
agttggttca gaagtacagt aattctgctc ttggtcatgt gaactgcacg
ataaaggaac tcaggcgcct cttcttagtt gatgatttag ttgattctct
45   gaag (SEQ ID NO:16)

20

It is, however, thought that fragments, derivatives or
variants of these specific domains will also give rise to
effective vaccines. Accordingly, a polypeptide domain
within the meaning of the invention may be a fragment of any
5   one of the four amino acid sequences given above, the
fragment preferably consisting of at least 15 contiguous
amino acids from said sequence, more preferably at least 17,
more preferably at least 20, 25, 30, 40, 50 or 60 amino
acids. For MAG, NogoN and TNR, the size of the fragment is
10   more preferably at least 80 amino acids, more preferably
100, 120, 140, 160 or 180 amino acids. For TNR and MAG, the
length is more preferably 200 amino acids. For MAG, the
length is more preferably 250, 300, 350, 400 or 450 amino
acids. The fragment will include one or more epitopes of
15   the specific domain whose sequence is given above, and will
retain the ability of the domain to raise an antibody
response in vivo. Thus, the fragment will be capable of
raising antibodies in vivo that cross-react with the
corresponding domain. Linear epitope mapping is a matter of
20   routine for the skilled person.

Similarly, a polypeptide domain within the meaning of the
invention may be a variant of any one amino acid sequence
(the reference amino acid sequence) given above. In this
25   context, a variant is a polypeptide having a sequence which
differs from the reference amino acid sequence, but which
includes a portion of at least 15 amino acids that has at
least 65% amino acid identity to a corresponding portion of
the reference sequence. Preferably the level of identity is
30   at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%.
Preferably the portion is at least 17 amino acids in length,
more preferred lengths are those given in the preceding
paragraph for the lengths of fragments. Again, the variant
will generally include one or more epitopes of the specific

21

domain whose sequence is given above, and will retain the
ability of the domain to raise an antibody response in vivo.
Thus, the variant will be capable of raising antibodies in
vivo that cross-react with the corresponding domain.

5

The invention also provides a composition comprising the
vector of the invention, formulated together with one or
more pharmaceutically acceptable ingredients for use as a
(therapeutic) vaccine. Preferably the composition is

10   formulated for administration by injection.

The invention further provides the vector of the invention,
for use in a method of treatment. The treatment may be of
CNS damage, especially SCI or stroke.

15

The invention also provides the use of the vector of the
invention in the manufacture of a medicament for the
treatment of CNS damage, especially SCI and stroke.

20   The invention also provides a method for treating CNS damage
in a patient, the method comprising administering a vector
of the invention to the patient as a therapeutic vaccine.

The vectors of the invention will typically be administered

25   by injection, although other vaccine delivery methods are
known in the art (such as oral or transdermal delivery and
"needle-free" injection) and may be used in accordance with
the invention. Injection will typically be intra-muscular.
While the blood-brain barrier normally provides an obstacle

30   to the passage of antibodies, CNS damage (especially injury,
e.g. external injury) will generally allow passage of
antibodies across the blood-brain barrier at the site of
damage. It is considered therefore that no special measures
are required to allow passage across the blood-brain barrier

22

of antibodies raised in response to vaccination with the
vector of the invention.

The invention further provides a polypeptide consisting
5   essentially of one or more polypeptide domains selected from
the group consisting of:
         (a) the N-terminal (NogoN) domain of Nogo-A;
         (b) the extracellular loop of Nogo-B (also present in
Nogo-A and Nogo-C);
10   (c) the third to fifth immunoglobulin-like repeats of
MAG; and
         (d) the EGF-like domain of TN-R.

Preferred features of the polypeptide are as defined above
15   for the polypeptide encoded by the vector.

The invention also provides a composition comprising the
polypeptide of the invention, formulated together with one
or more pharmaceutically acceptable ingredients for use as a
20   (therapeutic) vaccine. Preferably the composition is
formulated for administration by injection.

The invention further provides the polypeptide of the
invention, for use in a method of treatment. The treatment
25   may be of CNS damage, especially SCI or stroke.

The invention also provides the use of the polypeptide of
the invention in the manufacture of a medicament for the
treatment of CNS damage, especially SCI or stroke.
30
The invention also provides a method for treating CNS damage
in a patient, the method comprising administering a
polypeptide of the invention to the patient as a therapeutic
vaccine.

23

Administration is preferably as described for the vector.

The invention further provides analogous methods and uses of
5   antibodies capable of specifically binding to any one
domains (a)-(d), or preferably mixtures of antibodies
together capable of binding two, three or all four of
domains (a)-(d) for the treatment of CNS damage, especially
SCI or stroke. The antibodies will be administered directly
10   to the patient (preferably as for the peptides of the first
aspect, e.g. by direct injection into the CNS, into the site
of damage or into the cerebro-spinal fluid), instead of
administering a nucleic acid or polypeptide vaccine to
generate an antibody response. Antibody fragments capable
15   of specific binding are regarded to be antibodies for this
purpose.

Detailed description

20   Peptides

The term "peptide" is intended to refer to a molecule
consisting of several amino acids, adjacent pairs of amino
acids being linked by peptide bonds. A peptide bond has the
25   structure -CO-NH-. Amino acids may be naturally occurring
or non-naturally occurring. Terminal amino acids may
include terminal modifications. Naturally occurring chiral
amino acids (i.e. amino acids other than non-chiral glycine)
are of the L-isoform. Peptides of the invention, however,
30   may include or consist of amino acids of the D-isoform.
Such D-amino acids may be the D-isoforms of naturally
occurring L-amino acids, or may have no naturally occurring
L-isoform. The inclusion of D-amino acids in the peptides

of the invention may assist in reducing clearance of the
peptide in vivo.

Synthesis of peptides

5

Peptides may be generated wholly or partly by chemical
synthesis. The peptides of the invention can be readily
prepared according to well-established, standard liquid or,
preferably, solid-phase peptide synthesis methods, general
10   descriptions of which are broadly available (see, for
example, in J.M. Stewart and J.D. Young, Solid Phase Peptide
Synthesis, 2nd edition, Pierce Chemical Company, Rockford,
Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The
Practice of Peptide Synthesis, Springer Verlag, New York
15   (1984); and Applied Biosystems 430A Users Manual, ABI Inc.,
Foster City, California), or they may be prepared in
solution, by the liquid phase method or by any combination
of solid-phase, liquid phase and solution chemistry, e.g. by
first completing the respective peptide portion and then, if
20   desired and appropriate, after removal of any protecting
groups being present, by introduction of the residue X by
reaction of the respective carbonic or sulfonic acid or a
reactive derivative thereof.

25   Phage Display

Phage display is a recent and promising technique in
biomolecular engineering which can survey tens of millions
of short peptides for tight binding to an antibody receptor
30   or other binding protein. Phage display was firstly
described in 1985 (Smith et al, 1985) and is a selection
technique in which a peptide or protein is expressed as a
fusion with a coat protein of a bacteriophage, resulting in
display of the fused protein on the surface of the virion,

while the DNA encoding the fusion resides within the virion.
Phage display creates a physical linkage between a vast
library of random peptide sequences and DNA encoding each
sequence, allowing rapid identification of peptide ligands
5   for a variety of target molecules by an in vitro selection
process called panning (Scott et al, 1990; Arap et al 1998).

Interactions that involve particular protein domains (either
protein/protein or protein/non-protein interactions)
10   typically only need 8-12 amino acids, among which 5-8 amino
acids play a key role. Phage display libraries displaying
peptides of 6-10 amino acid residues have been successfully
used in a number of applications, including epitope mapping,
mapping protein-protein contacts, identification of peptide
15   mimetics of non-peptide ligands and design of novel vaccine
and new drugs (Scott et al, 1990; Cwirla et al, 1990; Devlin
et al 1990 Felici et al, 1991; Motti et al, 1994; Hong et
al, 1995; Arap et al, 1998; Nilsson et al, 2000).

20   Recent work has shown that a series of peptides isolated
using a broad range of enzymes as targets contained similar
amino acid sequences for each target and bound one or two
sites per target by competition analysis. Of 17 peptides
tested, 13 were found to be specific inhibitors of enzyme
25   function. Peptidic surrogate ligands identified using phage
display are preferentially targeted to a limited number of
sites that inhibit enzyme function (Hyde-DeRuyscher et al
2000).

30   Mimetics

Non-peptide "small molecules" are often preferred to
peptides for in vivo pharmaceutical use. Accordingly,

mimetics of the peptides of the invention may be designed
for pharmaceutical use.

The designing of mimetics to a known pharmaceutically active
5   compound is a known approach to the development of
pharmaceuticals based on a "lead" compound. This might be
desirable where the active compound is difficult or
expensive to synthesise or where it is unsuitable for a
particular method of administration, e.g. peptides are
10   unsuitable active agents for oral compositions as they tend
to be quickly degraded by proteases in the alimentary canal.
Mimetic design, synthesis and testing is generally used to
avoid randomly screening large number of molecules for a
target property.
15

There are several steps commonly taken in the design of a
mimetic from a compound having a given target property.
Firstly, the particular parts of the compound that are
critical and/or important in determining the target property
20   are determined. In the case of a peptide, this can be done
by systematically varying the amino acid residues in the
peptide, e.g. by substituting each residue in turn. Alanine
scans of peptide are commonly used to refine such peptide
motifs. These parts or residues constituting the active
25   region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is
modelled according to its physical properties, eg
stereochemistry, bonding, size and/or charge, using data
30   from a range of sources, eg spectroscopic techniques, X-ray
diffraction data and NMR. Computational analysis,
similarity mapping (which models the charge and/or volume of
a pharmacophore, rather than the bonding between atoms) and
other techniques can be used in this modelling process.

In a variant of this approach, the three-dimensional
structure of the ligand and its binding partner are
modelled. This can be especially useful where the ligand
5   and/or binding partner change conformation on binding,
allowing the model to take account of this in the design of
the mimetic.

A template molecule is then selected onto which chemical
10   groups which mimic the pharmacophore can be grafted. The
template molecule and the chemical groups grafted on to it
can conveniently be selected so that the mimetic is easy to
synthesise, is likely to be pharmacologically acceptable,
and does not degrade *in vivo*, while retaining the biological
15   activity of the lead compound. Alternatively, where the
mimetic is peptide based, further stability can be achieved
by cyclising the peptide, increasing its rigidity. The
mimetic or mimetics found by this approach can then be
screened to see whether they have the target property, or to
20   what extent they exhibit it. Further optimisation or
modification can then be carried out to arrive at one or
more final mimetics for in vivo or clinical testing.

Sequence identity

25

Percent (%) amino acid sequence identity with respect to a
reference sequence is defined as the percentage of amino
acid residues in a candidate sequence that are identical
with the amino acid residues in the reference sequence,
30   after aligning the sequences and introducing gaps, if
necessary, to achieve the maximum percent sequence identity,
and not considering any conservative substitutions as part
of the sequence identity. % identity values may be
determined by WU-BLAST-2 (Altschul et al., Methods in

Enzymology, 266:460-480 (1996)). WU-BLAST-2 uses several
search parameters, most of which are set to the default
values. The adjustable parameters are set with the following
values: overlap span = 1, overlap fraction = 0.125, word

5　threshold (T) = 11. A % amino acid sequence identity value
is determined by the number of matching identical residues
as determined by WU-BLAST-2, divided by the total number of
residues of the reference sequence (gaps introduced by WU-
BLAST-2 into the reference sequence to maximize the

10　alignment score being ignored), multiplied by 100.

Stringent conditions

Stringent conditions may be identified by those that: (1)

15　employ low ionic strength and high temperature for washing,
for example 0.015 M sodium chloride/0.0015 M sodium citrate/
0.1% sodium dodecyl sulfate at 50°C; (2) employ during
hybridization a denaturing agent, such as formamide, for
example 50% (v/v) formamide with 0.1% bovine serum

20　albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium
phosphate buffer at pH 6.5 with 760 mM sodium chloride, 75
mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x
SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium
phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x

25　Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml),
0.1% SDS, and 10% dextran sulfate at 42°C, with washes at
42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50%
formamide at 55°C, followed by a high-stringency wash
consisting of 0.1 x SSC containing EDTA at 55°C.

30

The subject

The subject to which the compositions and/or treatments of
the invention will be administered will be a mammal,

29

preferably an experimental animal such as a rodent (e.g. a
rabbit, rat or mouse), dog, cat, monkey or ape, or a farm
animal such as a cow, horse, sheep, pig or goat. More
preferably, the subject is human.

5

Generally, the subject will have CNS damage, usually CNS
injury, e.g. a head injury. More preferably, however, the
damage is to the spinal cord, e.g. SCI. In experimental
animals, the damage may be experimental. The CNS damage may
10   also result from a disease or disorder, e.g. epilepsy,
stroke, or a neurodegenerative condition, learning memory-
related condition and/or dementia such as Alzheimer's
disease or Parkinson's disease.

15   The treatments of the invention will generally be intended
for use in conjunction with other therapies, such as surgery
and/or rehabilitation.

Formulations

20

It is preferable to present the peptides, mimetics, and
vectors of the invention as pharmaceutical formulations
(e.g., composition, preparation, medicament) comprising at
least one active compound, as defined above, together with
25   one or more other pharmaceutically acceptable ingredients
well known to those skilled in the art, including, but not
limited to, pharmaceutically acceptable carriers, adjuvants,
excipients, buffers, preservatives and stabilisers. The
formulation may further comprise other active agents.

30

Thus, the present invention further provides a method of
making a pharmaceutical composition as previously defined,
the method comprising admixing at least one peptide or
vector of the invention together with one or more

30

pharmaceutically acceptable ingredients well known to those
skilled in the art, e.g., carriers, adjuvants, excipients,
etc..

5 The term "pharmaceutically acceptable" as used herein
   pertains to compounds, ingredients, materials, compositions,
   dosage forms, etc., which are, within the scope of sound
   medical judgment, suitable for use in contact with the
   tissues of the subject in question (e.g., human) without
10 excessive toxicity, irritation, allergic response, or other
   problem or complication, commensurate with a reasonable
   benefit/risk ratio. Each carrier, adjuvant, excipient, etc.
   must also be "acceptable" in the sense of being compatible
   with the other ingredients of the formulation.

15

   Suitable carriers, adjuvants, excipients, etc. can be found
   in standard pharmaceutical texts, for example Remington's
   Pharmaceutical Sciences, 20th Edition, 2000, pub.
   Lippincott, Williams & Wilkins; and Handbook of
20 Pharmaceutical Excipients, 2nd edition, 1994.

   Formulations may suitably be injectable formulations, e.g.
   in the form of aqueous, isotonic, pyrogen-free, sterile
   solutions, in which the active compound is dissolved. Such
25 liquids may additional contain other pharmaceutically
   acceptable ingredients, such as anti-oxidants, buffers,
   preservatives, stabilisers, bacteriostats, suspending
   agents, thickening agents, and solutes which render the
   formulation isotonic with the blood or cerebrospinal fluid.
30 Examples of suitable isotonic carriers for use in such
   formulations include Sodium Chloride Injection, Ringer's
   Solution, or Lactated Ringer's Injection. Typically, the
   concentration of the active compound in the liquid is from
   about 1 ng/ml to about 10 µg/ml, for example from about

10 ng/ml to about 1 µg/ml. The formulations may be
presented in unit-dose or multi-dose sealed containers, for
example, ampoules and vials, and may be stored in a freeze-
dried (lyophilised) condition requiring only the addition of
5   the sterile liquid carrier, for example water for
injections, immediately prior to use. Extemporaneous
injection solutions and suspensions may be prepared from
sterile powders, granules, and tablets.

10   Administration

Administration of the peptides of the invention will
generally be by injection, preferably directly into the CNS.
Injection may be directly into the site of damage, e.g. into
15   the site of damage. Alternatively, injection may be into
the cerebro-spinal fluid, typically near the site of injury.

Experimental work underlying the invention and embodiments
of the invention will now be described, by way of example
20   only, with reference to the accompanying **Figure 1**, which
shows the primers used to isolate the inhibitory domains
from the proteins MAG, TN-R and Nogo. Coding sequence (or
the complementary strand) is shown in bold. Start (Met) and
stop (***) codons are doubly underlined. Restriction sites
25   are underlined. SEQ ID NOS are as follows:

Primer	SEQ ID NO
MAG1	17
MAG2	18
TNR1	19
TNR2	20
NogoN1	21
NogoN2	22
Nogo66-1	23

Nogo66-2   24

Also shown schematically is the resultant construct (SEQ ID
NO:25) obtained by digestion and sequential connection of
5   the resultant PCR products. Restriction sites are
underlined. Ala-encoding codons are labelled. Coding
sequence for each inhibitory domain is represented by the
bracketed name of the domain. The stop codon is labelled
***.

10

Aspect 1, Example 1

Identification of Nogo domains responsible having inhibitory
15   **properties**

Repulsion assay

NG108 cells (mouse neuroblastoma – rat glioma hybrid cells,
20   commercially available from the American Tissue Type Culture
Collection, Manassas, VA, USA, under accession number ATCC
HB-12317) were plated on coated substrates using Nogo1-25,
Nogo1-50, Nogo1-66 and GST-Nogo-66 as well as GST as
control. Generation and purification of the recombinant
25   domains of Nogo, either alone or as fusion proteins with
GST, were performed as previously described (Xiao ZC et al,
1996).

NG108 cells were repelled significantly from Nogo1-50,
30   Nogo1-66, and GST-Nogo-66, but not Nogo1-25 and GST.

Neurite outgrowth assay

NG108 cells were plated on coated substrates using Nogo1-25,
Nogo1-50, Nogo1-66, and GST-Nogo-66 as well as GST as
control.

5   Neurite outgrowth of NG108 cells were inhibited
significantly from Nogo1-50, Nogo1-66, and GST Nogo-66, but
not Nogo1-25 and GST.

10   **Aspect 1, Example 2**

**Screening of novel short peptides against neuronal growth
inhibitory molecules TN-R, MAG, and Nogo through phage
display technology**

15

Purification of TN-R, MAG and Nogo from adult mouse brains
was performed by immumoaffinity chromatography as previously
described (Pesheva, P et al, 1989). Generation and
purification of the recombinant domains of TN-R, MAG, Nogo
20   as fusion proteins with GST were performed as previously
described (Xiao ZC et al, 1996). All these proteins were
used as phage binding targets for rapid screening, using the
Ph.D-7TM phage display peptide library kit (New England
Biolabs, Ltd) according to the manufacturer's instructions.

25

Briefly, a library of phages, displaying different peptide
sequences, was exposed to a plate coated with the target
protein. Unbound phage was washed away and specifically
bound phage was eluted by lowering pH. The eluted pool of
30   phage was amplified, and the process was repeated 3-4 times.

After 3-4 rounds of affinity selection, several specific
phage clones were isolated from 7-mer random peptide phage-
displayed libraries and identified by ELISA. The peptide-

34

encoding sequences of the specific phage clones were
determined by automated sequencing. The sequences of
peptides which bound specifically to the targeted proteins
were obtained.

5

The framework sequence of the phage coat protein, into which
a 7-mer peptide-encoding sequence is inserted in each phage,
is as follows:

10 TTA TTC GCA ATT CCT TTA GT<u>G GTA CC</u>T TTC TAT TCT CAC TCT (SEQ
    ID NO:26) … GGT GGA GGT T<u>CG GCC G</u>AA ACT GTT GAA AGT TGT (SEQ
    ID NO:27)

    … represents the site of insertion of the 7-mer peptide-
15 encoding sequence. A Kpn1 site is underlined, a Eag1 site
    is doubly underlined.

    Exemplary 7-mer peptide-encoding sequences are shown below.
    "n" refers to the number of isolated phages displaying a
20 peptide having the sequence shown (though there was of
    course some variability in the encoding nucleic acid
    sequences, owing to the degeneracy of the genetic code).
    "SIN" is an abbreviation for "SEQ ID NO." and refers to the
    peptide sequence. The SEQ ID NOS of the nucleic acid
25 sequences are 28-35.

Target	Exemplary nucleic acid	Peptide		SIN
Nogo-66	TAT CTG ACG CAG CCT CAG TCG	YLTQPQS	(n=43)	1
Nogo-66	GGT TCT CTG CCT CAT TCG CTG	GSLPHSL	(n=8)	2
TNR-EGFL	ACG CAG CTG TTT CCT CCT TAG	TQLFPPQ	(n=18)	3
TNR-EGFL	CAT TCT ATT CCT GAT AAT ATT	HSIPDNI	(n=3)	4
TNR-EGFL	CAT CAT ATG CCT CAT GAT AAG	HHMPHDK	(n=1)	5
TNR-EGFL	GGT TCT CTG CCT CAT TCG CTG	GSLPHSL	(n=1)	2
TNR-EGFL	TAT ACG ACG CCT CCG AGT CCT	YTTPPSP	(n=1)	6

35

MAG	TAT CTG ACG CAG CCT CAG TCG	YLTQPQS (n=19)	1
MAG	CAG CTT CCG CTT ATG CCT CGT	QLPLMPR (n=5)	7
MAG	ACG CAG CTG TTT CCT CCT CAG	TQLFPPQ (n=7)	3

5

Aspect 1, Example 3

In vitro assay for phages displaying peptides that reduce the inhibitory effect of Nogo, MAG and TN-R on neuronal
5   **adhesion**

Tissue culture petri dishes (Becton Dickinson) with a diameter of 3.5 cm were coated with methanol-solubilized nitrocellulose according to Lagenaur and Lemmon (1987) and
10   air-dried under a sterile hood. Then the petri dishes were incubated with PBS containing 5 µg/ml poly-DL-ornithine for 2 hours at 37°C. Subsequently, the dishes were washed three times with PBS and dried under a sterile hood.

15   Protein spots (1.5 µl of 5 µM GST, 5 µM GST-Nogo66, 100µM Nogo66, 100 µM Nogo1-50 or 100 µM Nogo1-25) were applied to the nitrocellulose-coated surfaces of the petri dishes and incubated for 2 hours at 37°C in a humidified atmosphere. Subsequently, the spots were washed three times with PBS.
20   The dishes were then flooded with PBS containing 2% heat-inactivated fatty acid-free BSA (Sigma) and incubated overnight to block residual non-specific protein binding sites.

25   Then, the dishes were washed with PBS and NG108 cells were plated in 2 ml of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum at a density of 2.5×10^5 cells/ml and incubated at 37°C in a humidified atmosphere. After 12 hours, the dishes were gently washed three times
30   with PBS and the cells fixed by flooding with PBS containing 2.5% glutaraldehyde. After fixation, cultures were stained with 0.5% toluidine blue (Sigma) in 2.5% sodium carbonate. The stained cultures were then washed three times with water and air-dried.

Cells adhering to the various spots were photographed and counted. All experiments were performed at least three times. Statistical analysis was carried out by Student test.

5   The level of significance was chosen as $p < 0.05$.

GST-Nogo66, Nogo66 and Nogo1-50 all displayed significantly reduced NG108 cell counts compared to GST; Nogo1-25 did not.

10   NG108 cells were plated on poly-DL-ornithine-treated tissue culture petri dish as single-cell suspensions. Specific phages that display the Nogo66-, MAG- and TNR-binding peptides identified above were added to the cell culture. Cells were maintained for 24 hours before fixation and

15   staining with toluidine blue. Cell adhesion was determined as previously described.

Phage displaying the peptide YLTQPQS (SEQ ID NO. 1) was able to block the inhibitory effects of Nogo-66 and MAG on NG108

20   cell binding; phage displaying the peptide TQLFPPQ (SEQ ID NO. 3) was able to block the inhibitory effect of TNR-EGFL on NG108 cell binding.

25   **Aspect 1, Example 4**

In vitro assay for peptides that reduce the inhibitory effect of Nogo, MAG and TN-R on neuronal adhesion

30   All the screened consensus peptides are synthesized using the solid-phase method by means of a Peptide Synthesizer Model 1000. Neurite outgrowth and growth cone repulsion assays are performed as described previously (Xiao ZC et al, 1997). Purified intact TN-R, MAG and Nogo and recombinant

domains of TN-R, MAG and Nogo are be coated as substrate, to
allow identification of the inhibitory domains of TN-R, MAG
and Nogo. In a further assay, the synthesized peptides are
mixed with the relevant protein/domain before coating, to
5   identify peptides that can neutralize the inhibitory effects
of TN-R, MAG and/or Nogo in vitro.

Aspect 1, Example 5

10

**In vivo test for peptides that can alleviate spinal cord
injury**

Those consensus short peptides that can neutralize the
15   inhibitory effects of TN-R, MAG and/or Nogo in vitro are
used in vivo to determine that they alleviate spinal cord
injury (Bregman et al, 1995). These peptides are injected
into spinal cord injured adult rat. Regeneration of axons
is assayed by immunohistochemistry by comparison with an
20   untreated control. Function improvement is also analyzed.

Aspect 1, Example 6

25   **Screening for candidate factors that can abolish the
inhibitory effects of TN-R, MAG, and/or Nogo**

Degenerate oligonucleotide sequences are determined for the
peptides that can neutralize the inhibitory effects of TN-R,
30   MAG and/or Nogo in vitro and/or in vivo (preferably both).
EST databases (preferably databases of CNS-derived ESTs) are
searched using the degenerate oligonucleotide sequences.
CNS cDNA libraries are screened using labelled degenerate
oligonucleotide probes. Hits represent candidate factors

that can abolish the inhibitory effects of TN-R, MAG and/or
Nogo. These candidate factors are purified as GST-fusion
protein, and the neutralizing effects are tested as
described previously for the peptides.

5

An alternative way to test the neutralizing effects in vivo
is to transplant candidate factor-transfected neurons to the
injured spinal cord in an experimental animal and observe
the neutralizing results.

10

New factors that can abolish the inhibitory effects of TN-R,
MAG and/or Nogo are therefore obtained, and are useful for
treating, or in the development of treatments for, CNS
damage, especially spinal injury.

15

Aspect 2, Example 1

Vector Construction

20   Nucleic acid encoding the following domains was obtained by
PCR: N-terminal and 66-Aa extracellular domain of Nogo-A,
third to fifth Ig like domain of MAG and the EGF like domain
of human TNR.

PCR primers were designed to include restriction enzyme
25   sites and to encode Ala linkers at the ends of the subcloned
sequences (see Fig. 1). Therefore, when the PCR products
were double digested by restriction enzymes and then
connected sequentially, DNA encoding the Ala linkers was
located between each pair of neighbouring domains (again,
30   see Fig. 1). The Ala linkers facilitate proper folding of
the domains.

Then, the sequentially connected DNA was double digested by
BamH I and XbaI and then inserted into a pcDNA 3.1 vector
(Invitrogen) to produce a recombinant vector. The
recombinant vector was verified by agarose gel and sequence
5   analysis.

Aspect 2, Example 2

Verification of the inhibitory functions of the recombinant
10 **vector in vitro**

Before the DNA vaccine can be injected into test animals, in
vitro testing must be carried out to make sure that the
vaccine can be expressed by mammalian cells and can be
15 secreted out of the cell to stimulate the immune system of
the host.

To check this, COS-1 cells were transfected with the
recombinant vector. After transient expression, the cells
20 and the medium were collected respectively and Western-Blot
was performed using antibodies to the inhibitory molecules
(Xiao et al, 1996) to verify that the recombinant protein is
secreted out the cell and can act as an antigen to stimulate
the host immune system when the vector is administered in
25 vivo as a vaccine.

Transfected cells were detected positive for MAG and TNR
antibodies compared to non-transfected cells.
Immunoreactivity for Nogo was detected in both transfected
30 and non-transfected cells.

48 hours after transient transfection with the recombinant
plasmid, the medium was collected and immunoprecipitated

41

with anti-NogoN or anti-TNR-EGFL antibody using protein A-
agarose. The precipitates were separated by 10% and 6% SDS-
PAGE and probed for Nogo and TNR, respectively. Each
precipitate revealed immunoreactivity for the corresponding
5   antibody, indicating that the protein is being secreted.

Aspect 2, Example 3

10 **Dot-blot of GST fusion proteins with pre-immune sera and
anti-sera**

Anti-sera were collected from Lewis rats after 2-month
vaccination with recombinant plasmid. A serial
15 concentration of GST-Nogo66, GST-NogoN, GST-TNR/EGFL, GST-
MAG Ig3-5 and GST were dotted as substrates and subsequently
blotted with sera. Anti-sera specially recognized GST
fusion proteins, but not GST. Pre-immune sera recognised
neither the fusion proteins, nor GST.

20

Aspect 2, Example 4

Trial of the recombinant vector as a DNA vaccine
25

To check the regeneration of the injured spinal cord after
immunization with the DNA vaccine, the recombinant DNA
vector is injected into a test animal. The anti-serum of
immunized animals is assayed to verify the immune response
30 after the injection of DNA vaccine.

A semi-transected model for spinal cord injury is used.
After a period of time, a morphological study of the injured

spinal cord neuron/axon, and a behavioral study of the
animals is performed.

(1)   Immunization and spinal cord lesioning

5

6 week-old female Lewis rats are immunized once weekly with
100μg of recombinant vector from the preceding examples.
pcDNA3.1 vector lacking the insertion is used as a control.
The vector is injected into the back of the rat. After the
10   rats have produced anti-serum, the spinal cords are
lesioned. The rats continue to receive twice monthly
immunizations for another 6 weeks.

The rats are anesthetized with Somnitol (1mg/20g body
15   weight), and a lower thoracic laminectomy is done (T9). The
dorsal half of the spinal cord is then cut with a pair of
microscissors to sever the corticospinal tracts. The depth
of each lesion, about 1mm, is estimated by a mark placed on
the tip of the micro-scissors. After a 6 week survival
20   period post lesion, the rats are anesthetized and a 5%
solution of WGA-HRP (wheat germ agglutinin-horseradish
peroxidase) is injected onto the sensory-motor cortex as
described (Li et al., 1996).   48 hours after injection of
WGA-HRP, the animals are sacrificed by intracardiac
25   perfusion, and the longitudinal cryostat section of the
spinal cord is reacted for HRP histochemistry as described
(Li et al., 1996).

(2)   Verification of the immuno-response of the immunized
30   animals

To verify that the antisera of the immunized animals have
the function of blocking the inhibitory effect of the
inhibitory domains on neurite outgrowth, a neurite growth

assay is used to test the serum of the immunized animals. A
4-well dish is first coated with solubilized nitrocellulose
and preincubated with 5µg/ml poly-L-lysine. The GST fusion
inhibitory domains or peptides are placed in the well as a
5   2µl drop and then incubated for 4 hrs at 37°C. These wells
are then incubated overnight at 4°C with the serum from
either control mice or mice immunized with the DNA vaccine.
The serum is removed, and postnatal day 10 rat cerebellar
neurons purified by Percoll density gradient centrifugation
10 are plated at a density of 1 x 10^6 cells per well. The cells
are cultured in serum-free chemically defined medium for 24
hrs, fixed with 4% paraformaldehyde, and stained with
Coomassie blue. Neurite length is measured using a
Universal Image I image analysis system. Data is analyzed
15 using the Student-Newman-Keul's test to determine
statistically significant differences (Li et. al., 1996).

(3)   Morphological study of the injured spinal cord

20 To check the regeneration of the transected axon, the rats
are perfused with 4% paraformaldehyde, and a 10µm thick
longitudinal cryostat section of the spinal cord is picked
up on gelatin-coated glass slides. The sections are
incubated with a biotinylated goat anti-rat antibody
25 overnight and then with streptavidin-conjugated fluorescein
for 1 hr, to detect circulating antibodies.

(4)   Functional testing of the animals

30 The functional recovery of the experimental rat is also
carried out. Contact placing response is tested by lightly
touching the dorsal aspect of the hind limb without causing
joint displacement. The ability of the animals to lift the
foot and place it onto the support surface is then assessed

in three to six repetitions. Greater than a 30% response
will be scored as positive (Kukel-Bagden et. al., 1993).

(5)   Safety testing

5

The safety of this vaccination approach is assessed by
administering the vaccine to test animals (unlesioned and
lesioned) and looking for adverse events, especially loss of
sensory or motor function and behavioral or cognitive
10   impairments.

CNS tissue is examined histologically to look for autoimmune
damage, e.g. by immunostaining using monoclonal anti-CNPase
(Sigma C5922, lot 71k4889). CNPase is a protein expressed
15   by oligodendrocytes, 2',3'-cyclic nucleotide 3'-
phosphodiesterase.

Although the foregoing invention has been described in some
20   detail by way of illustration and example for purposes of
clarity and understanding, it will be readily apparent to
those of ordinary skill in the art in light of the teachings
of this invention that certain changes and modifications may
be made thereto without departing from the spirit or scope
25   of the appended claims.

All documents cited herein are incorporated by reference in
their entirety and for all purposes.

30

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Claims

1.   A peptide, the amino acid sequence of which consists of
an amino acid sequence selected from the group consisting of:
       YLTQPQS (SEQ ID NO. 1);
       GSLPHSL (SEQ ID NO. 2);
       TQLFPPQ (SEQ ID NO. 3);
       HSIPDNI (SEQ ID NO. 4);
       HHMPHDK (SEQ ID NO. 5);
       YTTPPSP (SEQ ID NO. 6); and
       QLPLMPR (SEQ ID NO. 7).

2.   The peptide of claim 1, wherein the amino acid sequence
is selected from the group consisting of:
       YLTQPQS (SEQ ID NO. 1); and
       TQLFPPQ (SEQ ID NO. 3).

3.   A peptide up to 60 amino acids in length comprising an
amino acid sequence selected from the group consisting of:
       YLTQPQS (SEQ ID NO. 1);
       GSLPHSL (SEQ ID NO. 2);
       TQLFPPQ (SEQ ID NO. 3);
       HSIPDNI (SEQ ID NO. 4);
       HHMPHDK (SEQ ID NO. 5);
       YTTPPSP (SEQ ID NO. 6); and
       QLPLMPR (SEQ ID NO. 7),
wherein the peptide is capable of binding to Nogo, MAG and/or
TN-R.

4.   The peptide of claim 3, which comprises the amino acid
sequence YLTQPQS (SEQ ID NO. 1) or TQLFPPQ (SEQ ID NO. 3).

5.   A peptide up to 60 amino acids in length comprising an
amino acid sequence having at least 5 residues identical with

corresponding residues in an amino acid sequence selected from the group consisting of:

     YLTQPQS (SEQ ID NO. 1);

     GSLPHSL (SEQ ID NO. 2);

     TQLFPPQ (SEQ ID NO. 3);

     HSIPDNI (SEQ ID NO. 4);

     HHMPHDK (SEQ ID NO. 5);

     YTTPPSP (SEQ ID NO. 6); and

     QLPLMPR (SEQ ID NO. 7),

wherein the peptide is capable of binding to Nogo, MAG and/or TN-R.

6.   The peptide of claim 5, which has at least 5 residues identical with corresponding residues in an amino acid sequence selected from the group consisting of:

     YLTQPQS (SEQ ID NO. 1); and

     TQLFPPQ (SEQ ID NO. 3).

7.   The peptide of claim 5 or claim 6, wherein the number of identical residues is at least 6.

8.   The peptide of any one of claims 3 to 7, which is capable of binding to Nogo-66 and/or TNR-EGFL.

9.   The peptide of any one of claims 3 to 8, which is up to 40 amino acids in length.

10.   The peptide of claim 9, which is up to 20 amino acids in length.

11.   The peptide of claim 10, which is up to 10 amino acids in length.

12.   A composition comprising one or more peptides according
to any preceding claim, together with one or more
pharmaceutically acceptable ingredients.

13.   A peptide according to any one of claims 1 to 11 for use
in a method of treatment.

14.   The use of a peptide according to any one of claims 1 to
11 in the preparation of a medicament for the treatment of CNS
damage.

15.   A method for treating CNS damage, the method comprising
administering a peptide according to any one of claims 1 to 11
to a patient at or near a site of CNS damage in the patient.

16.   A method for treating spinal cord injury or stroke, the
method comprising administering to a patient a peptide having
an amino acid sequence that consists of an amino acid sequence
selected from the group consisting of:
      YLTQPQS (SEQ ID NO. 1); and
      TQLFPPQ (SEQ ID NO. 3),
by direct injection into a site of spinal cord injury or
stroke damage in the patient.

17.   The use of a peptide according to any one of claims 1 to
11 and/or a computer-generated model thereof, in the design of
a mimetic capable of binding to one or more of the neuronal
growth inhibitory molecules Nogo, MAG and/or TN-R.

18.   A method of designing a mimetic of a peptide as defined
in any one of claims 1 to 11, the mimetic being capable of
binding to one or more of the neuronal growth inhibitory
molecules Nogo, MAG and/or TN-R, said method comprising:

(i)   analysing a peptide as defined in any one of claims
1 t 11 that is capable of binding to one or more of the
neuronal growth inhibitory molecules Nogo, MAG and/or TN-R to
determine the amino acid residues essential and important for
the activity to define a pharmacophore; and

(ii) modelling the pharmacophore to design and/or screen
candidate mimetics having the biological activity.

19.   The use or method of claim 17 or claim 18, which includes
a step of assaying binding of a candidate mimetic to Nogo, MAG
and/or TN-R in vitro.

20.   The use or method of any one of claims 17 to 19, which
includes a step, having identified a candidate mimetic that is
capable of such in vitro binding, of optimizing the candidate
mimetic for in vivo use.

21.   The use or method of claim 20, wherein the optimised
mimetic is formulated together with one or more
pharmaceutically acceptable ingredients.

22.   A bacteriophage which expresses a fusion protein
consisting of a peptide and a bacteriophage coat protein, such
that the peptide is displayed on the surface of the
bacteriophage virion, wherein the peptide is as defined in any
one of claims 1 to 11.

23.   A screening method for peptides capable of binding to
Nogo, MAG and/or TN-R, the method comprising:
providing bacteriophages of claim 22, respectively
expressing different peptides; and
screening the bacteriophages for the ability to bind to
Nogo, MAG and/or TN-R.

24. The method of claim 23, wherein bacteriophages which are identified as being capable of binding to Nogo, MAG and/or TN-R, or the peptides they display, are then be screened for the ability to block the inhibitory effects of Nogo, MAG and/or TN-R on neuronal cell adhesion in an in vitro assay.

25. A method comprising all the steps of claim 24 and an additional step, following the identification of a peptide, or phage that displays a peptide, that is capable of blocking the inhibitory effects of Nogo, MAG and/or TN-R on neuronal cell adhesion in an in vitro assay, of formulating the peptide with one or more pharmaceutically acceptable ingredients for administration in vivo.

26. A method of searching for factors that are likely to reduce the inhibitory effect of TN-R, MAG and/or Nogo, the method comprising interrogating a sequence database to identify polypeptides, or nucleic acids that encode polypeptides, that comprise an amino acid sequence having at least 5 residues identical with corresponding residues in an amino acid sequence selected from the group consisting of:
    YLTQPQS (SEQ ID NO. 1);
    GSLPHSL (SEQ ID NO. 2);
    TQLFPPQ (SEQ ID NO. 3);
    HSIPDNI (SEQ ID NO. 4);
    HHMPHDK (SEQ ID NO. 5);
    YTTPPSP (SEQ ID NO. 6); and
    QLPLMPR (SEQ ID NO. 7).

27. A method of searching for factors that are likely to reduce the inhibitory effect of TN-R, MAG and/or Nogo, the method comprising screening a cDNA library with an oligonucleotide probe which is capable of hybridising under stringent conditions with a nucleic acid sequence that encodes

55

an amino acid sequence having at least 5 residues identical
with corresponding residues in an amino acid sequence selected
from the group consisting of:

      YLTQPQS (SEQ ID NO. 1);

      GSLPHSL (SEQ ID NO. 2);

      TQLFPPQ (SEQ ID NO. 3);

      HSIPDNI (SEQ ID NO. 4);

      HHMPHDK (SEQ ID NO. 5);

      YTTPPSP (SEQ ID NO. 6); and

      QLPLMPR (SEQ ID NO. 7).

28. The method of claim 26 or claim 27, which includes the
step, following the identification of a candidate polypeptide
or nucleic acid encoding the candidate polypeptide, of testing
the polypeptide for the ability to reduce the inhibitory
effect of TN-R, MAG and/or Nogo.

29. A method comprising all the steps of claim 28 and an
additional step, following the identification of a polypeptide
that is capable of blocking the inhibitory effects of Nogo,
MAG and/or TN-R on neuronal cell adhesion in an in vitro
assay, of formulating the polypeptide with one or more
pharmaceutically acceptable ingredients for administration in
vivo.

30. A nucleic acid vector comprising nucleic acid encoding
one or more polypeptide domains selected from the group
consisting of:

    (a) the N-terminal domain (NogoN) of Nogo-A, or a variant
or fragment thereof;

    (b) the extracellular loop (Nogo66) of Nogo-B, or a
variant or fragment thereof;

    (c) the third to fifth immunoglobulin-like repeats of
MAG, or a variant or fragment thereof; and

(d) the EGF-like domain of TN-R, or a variant or fragment thereof,

wherein said fragment comprises at least 15 contiguous amino acids from said domain, includes one or more epitopes of said domain and retains the ability to raise an antibody response in vivo, and

wherein said variant includes a portion of at least 15 amino acids that has at least 65% amino acid identity to a corresponding portion of said domain.

31. The vector of claim 30, wherein the nucleic acid encodes at least two of the domains.

32. The vector of claim 31, wherein the domains are expressed as a fusion polypeptide.

33. The vector of claim 32, wherein the domains are separated from one another by flexible linkers.

34. The vector of any one of claims 30 to 33 wherein, of the proteins Nogo A, Nogo B, TN-R and/or MAG, the vector is preferably capable of expressing substantially only the domains (a), (b), (c) and/or (d) recited in claim 30.

35. The vector of claim 34, wherein the vector is incapable of expressing other epitope-containing portions of the proteins.

36. The vector of claim 34 or claim 35, wherein, of the proteins Nogo A, Nogo B, TN-R and/or MAG, the vector preferably expresses no more than 20% of the protein lying outside domains (a) to (d) recited in claim 30.

37. The vector of any one of claims 30 to 36, wherein the domain (a) has the amino acid sequence of NogoN (1-185) (SEQ ID NO:10), domain (b) has the amino acid sequence of Nogo66 (823-888) (SEQ ID NO:11), domain (c) has the amino acid sequence of MAG (1-508) (SEQ ID NO:8), and domain (d) has the amino acid sequence of TNR (125-329) (SEQ ID NO:9).

38. The vector of claim 30, which encodes a polypeptide having the amino acid sequence $MAG(1-508)-Ala_n-TNR(125-329)-Ala_n-NogoN(1-185)-Ala_n-Nogo66(823-888)$ (SEQ ID NO:12), where Ala_n represents an polyalanine linker.

39. A composition comprising the vector of any one of claims 30 to 38, formulated together with one or more pharmaceutically acceptable ingredients for use as a therapeutic vaccine.

40. The vector of any one of claims 30 to 38, for use in a method of treatment.

41. Use of the vector of any one of claims 30 to 38 in the manufacture of a medicament for the treatment of CNS damage.

42. A method for treating CNS damage in a patient, the method comprising administering the vector of any one of claims 30 to 38 to the patient as a therapeutic vaccine.

43. A polypeptide consisting essentially of one or more polypeptide domains as defined in claim 30.

44. The polypeptide of claim 43, which is encoded by the vector of any one of claims 30-38.

45. A composition comprising the polypeptide of claim 43 or claim 44, formulated together with one or more pharmaceutically acceptable ingredients for use as a therapeutic vaccine.

46. The polypeptide of claim 43 or claim 44, for use in a method of treatment.

47. Use of the polypeptide of claim 43 or claim 44 in the manufacture of a medicament for the treatment of CNS damage.

48. A method for treating CNS damage in a patient, the method comprising administering the polypeptide of claim 43 or claim 44 to the patient as a therapeutic vaccine.

49. An antibody capable of specifically binding to any one of domains (a)-(d) as defined in claim 37, or a mixture of antibodies together capable of binding to two, three or all four of domains (a)-(d) as defined in claim 37, for use in a method of treatment.

50. Use of an antibody capable of specifically binding to any one of domains (a)-(d) as defined in claim 37, or a mixture of antibodies together capable of binding to two, three or all four of domains (a)-(d) as defined in claim 37, in the manufacture of a medicament for the treatment of CNS damage.

51. A method for treating CNS damage in a patient, the method comprising administering to the patient as a therapeutic vaccine an antibody capable of specifically binding to any one of domains (a)-(d) as defined in claim 37, or a mixture of antibodies together capable of binding to two, three or all four of domains (a)-(d) as defined in claim 37.

52. A composition comprising an antibody capable of specifically binding to any one of domains (a)-(d) as defined in claim 37, or a mixture of antibodies together capable of binding to two, three or all four of domains (a)-(d) as defined in claim 37, formulated together with one or more pharmaceutically acceptable ingredients for use as a therapeutic vaccine.

53. The composition of any one of claims 12, 39, 45 and 52, which is formulated for administration by injection.

54. The peptide of claim 13, the vector of claim 40, the polypeptide of claim 46 or the antibody of claim 49, wherein the treatment is the treatment of CNS damage.

55. The peptide of claim 13, the vector of claim 40, the polypeptide of claim 46 or the antibody of claim 49, wherein the treatment is the treatment of spinal cord injury or stroke.

56. The use of any one of claims 14, 41, 47 and 50, wherein the treatment is of spinal cord injury or stroke.

Fig. 1

PCR primers

MAG1: 5- *CG* GGA TCC ATG ATA TTC CTT ACC ACC CT
          ‾‾‾‾‾‾‾ ‾‾‾
          BamH I Met

MAG2: 5- *TCC* C CGC GGC TCG GTG TGC TCC CTG AAA
            ‾‾‾‾‾‾‾
            Sac II

TNR1: 5-*TCC* CC GCG GCA TGT CCA TGT GCC AGT TCA
           ‾‾‾‾‾‾‾
           Sac II

TNR2: 5- *TT* GC GGC CGC TGG AGG GGC AAC TGC TGA
            ‾‾‾‾‾‾‾
            Not I

NogoN1: 5-*TT* GCG GCC GCA ATG GAA GAC CTG GAC CAG TCT
             ‾‾‾‾‾‾‾‾‾‾
             Not I

NogoN2: 5-*AAA* C TGC AGC CAC TGA GCC CGA GGA GCC CCT
              ‾‾‾‾‾‾‾
              Pst I

Nogo66-1: 5-*AAA* CT GCA GCA AGG ATA TAC AAG GGT GT
               ‾‾‾‾‾‾‾
               Pst I

Nogo66-2: 5- *GC* TCT AGA TCA CTT CAG AGA ATC AAC TA
                ‾‾‾‾‾‾‾ ‾‾‾
                Xba I   ***

Construct resulting from sequentially connected PCR products

```
              Ala Ala Ala      Ala Ala Ala        Ala Ala Ala
5'-GGA TCC (MAG) GCC GCG GCA (TNR) GCG GCC GCA (NogoN) GCT GCA GCA
   ‾‾‾‾‾‾‾        ‾‾‾‾‾‾‾‾‾‾‾       ‾‾‾‾‾‾‾‾‾‾‾           ‾‾‾‾‾‾‾
   BamH I          Sac II            Not I               Pst I


        ***
  (Nogo66) TGA TCT AGA-3'
               ‾‾‾‾‾‾‾
               Xba I
```

SEQUENCE LISTING

<110> Singapore General Hospital Pte Ltd
      Xiao, Zhi-Cheng

<120> Central nervous system damage

<130> CMD/FP6186654

<150> US 60/431,620
<151> 2002-12-06

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Met Ile Phe Leu Thr Thr Leu Pro Leu Phe Trp Ile Met Ile Ser Ala
1               5                   10                  15
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Ser Arg Gly Gly His Trp Gly Ala Trp Met Pro Ser Ser Ile Ser Ala
        20              25              30

Phe Glu Gly Thr Cys Val Ser Ile Pro Cys Arg Phe Asp Phe Pro Asp
        35              40              45

Glu Leu Arg Pro Ala Val Val His Gly Val Trp Tyr Phe Asn Ser Pro
    50              55              60

Tyr Pro Lys Asn Tyr Pro Pro Val Val Phe Lys Ser Arg Thr Gln Val
65              70              75              80

Val His Glu Ser Phe Gln Gly Arg Ser Arg Leu Leu Gly Asp Leu Gly
            85              90              95

Leu Arg Asn Cys Thr Leu Leu Leu Ser Thr Leu Ser Pro Glu Leu Gly
            100             105             110

Gly Lys Tyr Tyr Phe Arg Gly Asp Leu Gly Gly Tyr Asn Gln Tyr Thr
        115             120             125

Phe Ser Glu His Ser Val Leu Asp Ile Ile Asn Thr Pro Asn Ile Val
    130             135             140

Val Pro Pro Glu Val Val Ala Gly Thr Glu Val Glu Val Ser Cys Met
145             150             155             160

Val Pro Asp Asn Cys Pro Glu Leu Arg Pro Glu Leu Ser Trp Leu Gly
            165             170             175

His Glu Gly Leu Gly Glu Pro Thr Val Leu Gly Arg Leu Arg Glu Asp
            180             185             190

Glu Gly Thr Trp Val Gln Val Ser Leu Leu His Phe Val Pro Thr Arg
        195             200             205

Glu Ala Asn Gly His Arg Leu Gly Cys Gln Ala Ala Phe Pro Asn Thr
    210             215             220

Thr Leu Gln Phe Glu Gly Tyr Ala Ser Leu Asp Val Lys Tyr Pro Pro
225             230             235             240

Val Ile Val Glu Met Asn Ser Ser Val Glu Ala Ile Glu Gly Ser His
            245             250             255

Val Ser Leu Leu Cys Gly Ala Asp Ser Asn Pro Pro Pro Leu Leu Thr
```

```
                    260                    265                    270

     Trp Met Arg Asp Gly Met Val Leu Arg Glu Ala Val Ala Glu Ser Leu
         275                    280                    285


     Tyr Leu Asp Leu Glu Glu Val Thr Pro Ala Glu Asp Gly Ile Tyr Ala
         290                    295                    300


     Cys Leu Ala Glu Asn Ala Tyr Gly Gln Asp Asn Arg Thr Val Glu Leu
     305                    310                    315                    320


     Ser Val Met Tyr Ala Pro Trp Lys Pro Thr Val Asn Gly Thr Val Val
                     325                    330                    335


     Ala Val Glu Gly Glu Thr Val Ser Ile Leu Cys Ser Thr Gln Ser Asn
                 340                    345                    350


     Pro Asp Pro Ile Leu Thr Ile Phe Lys Glu Lys Gln Ile Leu Ala Thr
                 355                    360                    365


     Val Ile Tyr Glu Ser Gln Leu Gln Leu Glu Leu Pro Ala Val Thr Pro
         370                    375                    380


     Glu Asp Asp Gly Glu Tyr Trp Cys Val Ala Glu Asn Gln Tyr Gly Gln
     385                    390                    395                    400


     Arg Ala Thr Ala Phe Asn Leu Ser Val Glu Phe Ala Pro Ile Ile Leu
                     405                    410                    415


     Leu Glu Ser His Cys Ala Ala Ala Arg Asp Thr Val Gln Cys Leu Cys
                 420                    425                    430


     Val Val Lys Ser Asn Pro Glu Pro Ser Val Ala Phe Glu Leu Pro Ser
             435                    440                    445


     Arg Asn Val Thr Val Asn Glu Thr Glu Arg Glu Phe Val Tyr Ser Glu
         450                    455                    460


     Arg Ser Gly Leu Leu Leu Thr Ser Ile Leu Thr Leu Arg Gly Gln Ala
     465                    470                    475                    480


     Gln Ala Pro Pro Arg Val Ile Cys Thr Ser Arg Asn Leu Tyr Gly Thr
                     485                    490                    495


     Gln Ser Leu Glu Leu Pro Phe Gln Gly Ala His Arg
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            20                  25                  30

Ala Asn Cys Cys Gln Glu Ser Ala Ala Thr Gly Gln Leu Asp Tyr Ile
        35                  40                  45

Pro His Cys Ser Gly His Gly Asn Phe Ser Phe Glu Ser Cys Gly Cys
    50                  55                  60

Ile Cys Asn Glu Gly Trp Phe Gly Lys Asn Cys Ser Glu Pro Tyr Cys
65                  70                  75                  80

Pro Leu Gly Cys Ser Ser Arg Gly Val Cys Val Asp Gly Gln Cys Ile
            85                  90                  95

Cys Asp Ser Glu Tyr Ser Gly Asp Asp Cys Ser Glu Leu Arg Cys Pro
            100                 105                 110

Thr Asp Cys Ser Ser Arg Gly Leu Cys Val Asp Gly Glu Cys Val Cys
            115                 120                 125

Glu Glu Pro Tyr Thr Gly Glu Asp Cys Arg Glu Leu Arg Cys Pro Gly
    130                 135                 140

Asp Cys Ser Gly Lys Gly Arg Cys Ala Asn Gly Thr Cys Leu Cys Glu
145                 150                 155                 160

Glu Gly Tyr Val Gly Glu Asp Cys Gly Gln Arg Gln Cys Leu Asn Ala
            165                 170                 175

Cys Ser Gly Arg Gly Gln Cys Glu Glu Gly Leu Cys Val Cys Glu Glu
            180                 185                 190

Gly Tyr Gln Gly Pro Asp Cys Ser Ala Val Ala Pro Pro
        195                 200                 205

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1               5                  10                 15


Pro Arg Pro Gln Pro Ala Phe Lys Tyr Gln Phe Val Arg Glu Pro Glu
            20                  25                 30


Asp Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Asp Glu Asp Glu Asp
        35                  40                 45


Leu Glu Glu Leu Glu Val Leu Glu Arg Lys Pro Ala Ala Gly Leu Ser
    50                  55          .       60


Ala Ala Pro Val Pro Thr Ala Pro Ala Ala Gly Ala Pro Leu Met Asp
65                  70                  75                 80


Phe Gly Asn Asp Phe Val Pro Pro Ala Pro Arg Gly Pro Leu Pro Ala
                85                  90                 95


Ala Pro Pro Val Ala Pro Glu Arg Gln Pro Ser Trp Asp Pro Ser Pro
            100                 105                110


Val Ser Ser Thr Val Pro Ala Pro Ser Pro Leu Ser Ala Ala Ala Val
        115                 120                 125


Ser Pro Ser Lys Leu Pro Glu Asp Asp Glu Pro Pro Ala Arg Pro Pro
    130                 135                 140


Pro Pro Pro Pro Ala Ser Val Ser Pro Gln Ala Glu Pro Val Trp Thr
145                 150                 155                160


Pro Pro Ala Pro Ala Pro Ala Ala Pro Pro Ser Thr Pro Ala Ala Pro
                165                 170                 175


Lys Arg Arg Gly Ser Ser Gly Ser Val
                180             185


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<213>    Homo sapiens

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Arg Ile Tyr Lys Gly Val Ile Gln Ala Ile Gln Lys Ser Asp Glu Gly
1               5                  10                 15
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His Pro Phe Arg Ala Tyr Leu Glu Ser Glu Val Ala Ile Ser Glu Glu
            20              25              30

Leu Val Gln Lys Tyr Ser Asn Ser Ala Leu Gly His Val Asn Cys Thr
        35              40              45

Ile Lys Glu Leu Arg Arg Leu Phe Leu Val Asp Asp Leu Val Asp Ser
    50              55              60

Leu Lys
65
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Ser Arg Gly Gly His Trp Gly Ala Trp Met Pro Ser Ser Ile Ser Ala
            20              25              30

Phe Glu Gly Thr Cys Val Ser Ile Pro Cys Arg Phe Asp Phe Pro Asp
        35              40              45

Glu Leu Arg Pro Ala Val Val His Gly Val Trp Tyr Phe Asn Ser Pro
    50              55              60
```

```
Tyr Pro Lys Asn Tyr Pro Pro Val Val Phe Lys Ser Arg Thr Gln Val
65              70              75              80


Val His Glu Ser Phe Gln Gly Arg Ser Arg Leu Leu Gly Asp Leu Gly
                85              90              95


Leu Arg Asn Cys Thr Leu Leu Ser Thr Leu Ser Pro Glu Leu Gly
            100             105             110


Gly Lys Tyr Tyr Phe Arg Gly Asp Leu Gly Gly Tyr Asn Gln Tyr Thr
        115             120             125


Phe Ser Glu His Ser Val Leu Asp Ile Ile Asn Thr Pro Asn Ile Val
    130             135             140


Val Pro Pro Glu Val Val Ala Gly Thr Glu Val Glu Val Ser Cys Met
145             . 150           155             160


Val Pro Asp Asn Cys Pro Glu Leu Arg Pro Glu Leu Ser Trp Leu Gly
            165             170             175


His Glu Gly Leu Gly Glu Pro Thr Val Leu Gly Arg Leu Arg Glu Asp
        180             185             190


Glu Gly Thr Trp Val Gln Val Ser Leu Leu His Phe Val Pro Thr Arg
        195             200             205


Glu Ala Asn Gly His Arg Leu Gly Cys Gln Ala Ala Phe Pro Asn Thr
    210             215             220


Thr Leu Gln Phe Glu Gly Tyr Ala Ser Leu Asp Val Lys Tyr Pro Pro
225             230             235             240


Val Ile Val Glu Met Asn Ser Ser Val Glu Ala Ile Glu Gly Ser His
            245             . 250           255


Val Ser Leu Leu Cys Gly Ala Asp Ser Asn Pro Pro Pro Leu Leu Thr
            260             265             270


Trp Met Arg Asp Gly Met Val Leu Arg Glu Ala Val Ala Glu Ser Leu
        275             280             285


Tyr Leu Asp Leu Glu Glu Val Thr Pro Ala Glu Asp Gly Ile Tyr Ala
    290             295             300


Cys Leu Ala Glu Asn Ala Tyr Gly Gln Asp Asn Arg Thr Val Glu Leu
305             310             315             320
```

```
Ser Val Met Tyr Ala Pro Trp Lys Pro Thr Val Asn Gly Thr Val Val
            325             330             335

Ala Val Glu Gly Glu Thr Val Ser Ile Leu Cys Ser Thr Gln Ser Asn
            340             345             350

Pro Asp Pro Ile Leu Thr Ile Phe Lys Glu Lys Gln Ile Leu Ala Thr
        355             360             365

Val Ile Tyr Glu Ser Gln Leu Gln Leu Glu Leu Pro Ala Val Thr Pro
    370             375             380

Glu Asp Asp Gly Glu Tyr Trp Cys Val Ala Glu Asn Gln Tyr Gly Gln
385             390             395             400

Arg Ala Thr Ala Phe Asn Leu Ser Val Glu Phe Ala Pro Ile Ile Leu
            405             410             415

Leu Glu Ser His Cys Ala Ala Ala Arg Asp Thr Val Gln Cys Leu Cys
            420             425             430

Val Val Lys Ser Asn Pro Glu Pro Ser Val Ala Phe Glu Leu Pro Ser
        435             440             445

Arg Asn Val Thr Val Asn Glu Thr Glu Arg Glu Phe Val Tyr Ser Glu
    450             455             460

Arg Ser Gly Leu Leu Leu Thr Ser Ile Leu Thr Leu Arg Gly Gln Ala
465             470             475             480

Gln Ala Pro Pro Arg Val Ile Cys Thr Ser Arg Asn Leu Tyr Gly Thr
            485             490             495

Gln Ser Leu Glu Leu Pro Phe Gln Gly Ala His Arg Ala Ala Ala Cys
            500             505             510

Pro Cys Ala Ser Ser Ala Gln Val Leu Gln Glu Leu Leu Ser Arg Ile
        515             520             525

Glu Met Leu Glu Arg Glu Val Ser Val Leu Arg Asp Gln Cys Asn Ala
    530             535             540

Asn Cys Cys Gln Glu Ser Ala Ala Thr Gly Gln Leu Asp Tyr Ile Pro
545             550             555             560
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```
His Cys Ser Gly His Gly Asn Phe Ser Phe Glu Ser Cys Gly Cys Ile
            565             570             575

Cys Asn Glu Gly Trp Phe Gly Lys Asn Cys Ser Glu Pro Tyr Cys Pro
            580             585             590

Leu Gly Cys Ser Ser Arg Gly Val Cys Val Asp Gly Gln Cys Ile Cys
            595             600             605

Asp Ser Glu Tyr Ser Gly Asp Asp Cys Ser Glu Leu Arg Cys Pro Thr
    610             615             620

Asp Cys Ser Ser Arg Gly Leu Cys Val Asp Gly Glu Cys Val Cys Glu
625             630             635             640

Glu Pro Tyr Thr Gly Glu Asp Cys Arg Glu Leu Arg Cys Pro Gly Asp
            645             650             655

Cys Ser Gly Lys Gly Arg Cys Ala Asn Gly Thr Cys Leu Cys Glu Glu
            660             665             670

Gly Tyr Val Gly Glu Asp Cys Gly Gln Arg Gln Cys Leu Asn Ala Cys
            675             680             685

Ser Gly Arg Gly Gln Cys Glu Glu Gly Leu Cys Val Cys Glu Glu Gly
    690             695             700

Tyr Gln Gly Pro Asp Cys Ser Ala Val Ala Pro Pro Ala Ala Ala Met
705             710             715             720

Glu Asp Leu Asp Gln Ser Pro Leu Val Ser Ser Ser Asp Ser Pro Pro
            725             730             735

Arg Pro Gln Pro Ala Phe Lys Tyr Gln Phe Val Arg Glu Pro Glu Asp
            740             745             750

Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Asp Glu Asp Glu Asp Leu
            755             760             765

Glu Glu Leu Glu Val Leu Glu Arg Lys Pro Ala Ala Gly Leu Ser Ala
    770             775             780

Ala Pro Val Pro Thr Ala Pro Ala Ala Gly Ala Pro Leu Met Asp Phe
785             790             795             800

Gly Asn Asp Phe Val Pro Pro Ala Pro Arg Gly Pro Leu Pro Ala Ala
            805             810             815
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```
Pro Pro Val Ala Pro Glu Arg Gln Pro Ser Trp Asp Pro Ser Pro Val
        820                 825                 830

Ser Ser Thr Val Pro Ala Pro Ser Pro Leu Ser Ala Ala Ala Val Ser
        835                 840                 845

Pro Ser Lys Leu Pro Glu Asp Asp Glu Pro Pro Ala Arg Pro Pro Pro
    850                 855                 860

Pro Pro Pro Ala Ser Val Ser Pro Gln Ala Glu Pro Val Trp Thr Pro
865                 870                 875                 880

Pro Ala Pro Ala Pro Ala Ala Pro Pro Ser Thr Pro Ala Ala Pro Lys
                885                 890                 895

Arg Arg Gly Ser Ser Gly Ser Val Ala Ala Ala Arg Ile Tyr Lys Gly
            900                 905                 910

Val Ile Gln Ala Ile Gln Lys Ser Asp Glu Gly His Pro Phe Arg Ala
        915                 920                 925

Tyr Leu Glu Ser Glu Val Ala Ile Ser Glu Glu Leu Val Gln Lys Tyr
    930                 935                 940

Ser Asn Ser Ala Leu Gly His Val Asn Cys Thr Ile Lys Glu Leu Arg
945                 950                 955                 960

Arg Leu Phe Leu Val Asp Asp Leu Val Asp Ser Leu Lys
            965                 970
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<210> 13
<211> 1524
<212> DNA
<213> Rattus norvegicus

<400> 13

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cactggggtg cctggatgcc ctcgtccatc tcagccttcg agggcacgtg tgtctccatc    120

ccctgccgtt tcgacttccc ggatgagctc agaccggctg tggtacatgg cgtctggtat    180

ttcaacagtc cctaccccaa gaactacccg ccagtggtct tcaagtcccg cacacaagtg    240

gtccacgaga gcttccaggg ccgtagccgc ctgttgggag acctgggcct acgaaactgc    300

accctgcttc tcagcacgct gagccctgag ctgggaggga aatactattt ccgaggtgac    360

ctgggcggct acaaccagta caccttctcg gagcacagcg tcctggacat catcaacacc    420
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cccaacatcg tggtgccccc agaagtggtg gcaggaacgg aagtagaggt cagctgcatg    480

gtgccggaca actgcccaga gctgcgccct gagctgagct ggctgggcca cgaggggcta    540

ggggagccca ctgttctggg tcggctgcgg gaggatgaag gcacctgggt gcaggtgtca    600

ctgctacact tcgtgcctac tagagaggcc aacggccacc gtctgggctg tcaggctgcc    660

ttccccaaca ccaccttgca gttcgagggt tacgccagtc tggacgtcaa gtaccccccg    720

gtgattgtgg agatgaattc ctctgtggag gccattgagg gctcccacgt cagcctgctc    780

tgtggggctg acagcaaccc gccaccgctg ctgacttgga tgcgggatgg gatggtgttg    840

agggaggcag ttgctgagag cctgtacctg gatctggagg aggtgacccc agcagaggac    900

ggcatctatg cttgcctggc agagaatgcc tatggccagg acaaccgcac ggtggagctg    960

agcgtcatgt atgcaccttg gaagcccaca gtgaatggga cggtggtggc ggtagagggg   1020

gagacagtct ccatcctgtg ttccacacag agcaacccgg accctattct caccatcttc   1080

aaggagaagc agatcctggc cacggtcatc tatgagagtc agctgcagct ggaactccct   1140

gcagtgacgc ccgaggacga tggggagtac tggtgtgtag ctgagaacca gtatggccag   1200

agagccaccg ccttcaacct gtctgtggag tttgctccca taatccttct ggaatcgcac   1260

tgtgcagcgg ccagagacac cgtgcagtgc ctgtgtgtgg taaaatccaa cccggaaccc   1320

tccgtggcct ttgagctgcc ttcccgcaac gtgactgtga acgagacaga gagggagttt   1380

gtgtactcag agcgcagcgg cctcctgctc accagcatcc tcacgctccg gggtcaggcc   1440

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ctgcctttcc agggagcaca ccga                                          1524
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<210> 14
<211> 615
<212> DNA
<213> Homo sapiens

<400> 14
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gccacaggac aactggacta tatccctcac tgcagtggcc acggcaactt tagctttgag    180

tcctgtggct gcatctgcaa cgaaggctgg tttggcaaga attgctcgga gccctactgc    240

ccgctgggtt gctccagccg ggggggtgtgt gtggatggcc agtgcatctg tgacagcgaa    300

tacagcgggg atgactgttc cgaactccgg tgcccaacag actgcagctc ccggggggctc    360

tgcgtggacg gggagtgtgt ctgtgaagag ccctacactg gcgaggactg cagggaactg    420

aggtgccctg gggactgttc ggggaagggg agatgtgcca acggtacctg tttatgcgag    480

gagggctacg ttggtgagga ctgcggccag cggcagtgtc tgaatgcctg cagtgggcga    540
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ggacaatgtg aggaggggct ctgcgtctgt gaagagggct accagggccc tgactgctca   600

gcagttgccc ctcca   615

<210>   15
<211>   555
<212>   DNA
<213>   Homo sapiens

<400>   15
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cccgcgttca agtaccagtt cgtgagggag cccgaggacg aggaggaaga agaggaggag   120

gaagaggagg acgaggacga agacctggag gagctggagg tgctggagag gaagcccgcc   180

gccgggctgt ccgcggcccc agtgcccacc gccctgccg ccggcgcgcc cctgatggac   240

ttcggaaatg acttcgtgcc gccggcgccc cggggacccc tgccggccgc tcccccgtc   300

gccccggagc ggcagccgtc ttgggaccg agcccggtgt cgtcgaccgt gcccgcgcca   360

tccccgctgt ctgctgccgc agtctcgccc tccaagctcc ctgaggacga cgagcctccg   420

gcccggcctc ccctcctcc cccggccagc gtgagccccc aggcagagcc cgtgtggacc   480

ccgccagccc cggctcccgc cgcgcccccc tccaccccgg ccgcgcccaa gcgcaggggc   540

tcctcgggct cagtg   555

<210>   16
<211>   198
<212>   DNA
<213>   Homo sapiens

<400>   16
aggatataca agggtgtgat ccaagctatc cagaaatcag atgaaggcca cccattcagg   60

gcatatctgg aatctgaagt tgctatatct gaggagttgg ttcagaagta cagtaattct   120

gctcttggtc atgtgaactg cacgataaag gaactcaggc gcctcttctt agttgatgat   180

ttagttgatt ctctgaag   198

<210>   17
<211>   28
<212>   DNA
<213>   Artificial sequence

<220>
<223>   PCR primer MAG1

<400>   17
cgggatccat gatattcctt accaccct   28

<210>   18
<211>   28

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<212>  DNA
<213>  Artificial sequence

<220>
<223>  PCR primer MAG2

<400>  18
tccccgcggc tcggtgtgct ccctggaa                                           28


<210>  19
<211>  29
<212>  DNA
<213>  Artificial sequence

<220>
<223>  PCR primer TNR1

<400>  19
tccccgcggc atgtccatgt gccagttca                                          29


<210>  20
<211>  28
<212>  DNA
<213>  Artificial sequence

<220>
<223>  PCR primer TNR2

<400>  20
ttgcggccgc tggaggggca actgctga                                           28


<210>  21
<211>  32
<212>  DNA
<213>  Artificial sequence

<220>
<223>  PCR primer NogoN1

<400>  21
ttgcggccgc aatggaagac ctggaccagt ct                                      32


<210>  22
<211>  31
<212>  DNA
<213>  Artificial sequence

<220>
<223>  PCR primer NogoN2

<400>  22
aaactgcagc cactgagccc gaggagcccc t                                       31


<210>  23
<211>  28
<212>  DNA
<213>  Artificial sequence
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<220>
<223>  PCR primer Nogo66-1

<400>  23
aaactgcagc aaggatatac aagggtgt                                          28


<210>  24
<211>  28
<212>  DNA
<213>  Artificial sequence

<220>
<223>  PCR primer Nogo66-2

<400>  24
gctctagatc acttcagaga atcaacta                                         28


<210>  25
<211>  2934
<212>  DNA
<213>  Artificial sequence

<220>
<223>  Construct resulting from sequentially connected PCR products

<400>  25
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tccatcccct gccgtttcga cttcccggat gagctcagac cggctgtggt acatggcgtc    180

tggtatttca acagtcccta ccccaagaac tacccgccag tggtcttcaa gtcccgcaca    240

caagtggtcc acgagagctt ccagggccgt agccgcctgt gggagacct gggcctacga     300

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aacaccccca acatcgtggt gcccccagaa gtggtggcag gaacggaagt agaggtcagc    480

tgcatggtgc cggacaactg cccagagctg cgccctgagc tgagctggct gggccacgag    540

gggctagggg agcccactgt tctgggtcgg ctgcgggagg atgaaggcac ctgggtgcag    600

gtgtcactgc tacacttcgt gcctactaga gaggccaacg gccaccgtct gggctgtcag    660

gctgccttcc ccaacaccac cttgcagttc gagggttacg ccagtctgga cgtcaagtac    720

cccccggtga ttgtggagat gaattcctct gtggaggcca ttgagggctc ccacgtcagc    780

ctgctctgtg gggctgacag caacccgcca ccgctgctga cttggatgcg ggatgggatg    840

gtgttgaggg aggcagttgc tgagagcctg tacctggatc tggaggaggt gacccagca     900

gaggacggca tctatgcttg cctggcagag aatgcctatg ccaggacaa ccgcacggtg      960

gagctgagcg tcatgtatgc accttggaag cccacagtga atgggacggt ggtggcggta   1020
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gaggggggaga cagtctccat cctgtgttcc acacagagca acccggaccc tattctcacc   1080

atcttcaagg agaagcagat cctggccacg gtcatctatg agagtcagct gcagctggaa  . 1140

ctccctgcag tgacgcccga ggacgatggg gagtactggt gtgtagctga gaaccagtat   1200

ggccagagag ccaccgcctt caacctgtct gtggagtttg ctcccataat ccttctggaa   1260

tcgcactgtg cagcggccag agacaccgtg cagtgcctgt gtgtggtaaa atccaacccg   1320

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caggcccaag ccccacccccg cgtcatttgt acctccagga acctctacgg cacccagagc   1500

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ggggtgtgtg tggatggcca gtgcatctgt gacagcgaat acagcgggga tgactgttcc   1860

gaactccggt gcccaacaga ctgcagctcc cgggggctct gcgtggacgg ggagtgtgtc   1920

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gggaagggga gatgtgccaa cggtacctgt ttatgcgagg agggctacgt tggtgaggac   2040

tgcggccagc ggcagtgtct gaatgcctgc agtgggcgag acaatgtgga ggaggggctc   2100

tgcgtctgtg aagagggcta ccagggccct gactgctcag cagttgcccc tccagcggcc   2160

gcaatggaag acctggacca gtctcctctg gtctcgtcct cggacagccc accccggccg   2220

cagcccgcgt tcaagtacca gttcgtgagg gagcccgagg acgaggagga agaagaggag   2280

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accccgccag ccccggctcc cgccgcgccc ccctccaccc cggccgcgcc caagcgcagg    2700

ggctcctcgg gctcagtggc tgcagcaagg atatacaagg gtgtgatcca agctatccag    2760

aaatcagatg aaggccaccc attcagggca tatctggaat ctgaagttgc tatatctgag    2820

gagttggttc agaagtacag taattctgct cttggtcatg tgaactgcac gataaaggaa   2880
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ctcaggcgcc tcttcttagt tgatgattta gttgattctc tgaagtgatc taga          2934


<210>  26
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<212>  DNA
<213>  M13 coliphage

<400>  26
ttattcgcaa ttcctttagt ggtacctttc tattctcact ct                        42


<210>  27
<211>  33
<212>  DNA
<213>  M13 coliphage

<400>  27
ggtggaggtt cggccgaaac tgttgaaagt tgt                                   33


<210>  28
<211>  21
<212>  DNA
<213>  Artificial sequence

<220>
<223>  Exemplary 7-mer peptide-encoding sequence

<400>  28
tatctgacgc agcctcagtc g                                                21


<210>  29
<211>  21
<212>  DNA
<213>  Artificial sequence

<220>
<223>  Exemplary 7-mer peptide-encoding sequence

<400>  29
ggttctctgc ctcattcgct g                                                21


<210>  30
<211>  21
<212>  DNA
<213>  Artificial sequence

<220>
<223>  Exemplary 7-mer peptide-encoding sequence

<400>  30
acgcagctgt ttcctcctta g                                                21


<210>  31
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<212>  DNA
<213>  Artificial sequence
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<220>
<223>   Exemplary 7-mer peptide-encoding sequence

<400>   31
cattctattc ctgataatat t   21

<210>   32
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<210>   33
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<400>   33
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<210>   34
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<220>
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